PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF INDIAN MEDICINAL PLANTS

ABSTRACT

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

RAJINDER SINGH

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1996

ABSTRACT

The constituents of the leaves of *Calophyllum inophyllum* (Guttifereae) were isolated and structural elucidation was done by chemical and spectral methods (UV, IR, MS, ^{1}H NMR and ^{13}C NMR). The following new constituents were isolated and characterized.

(1) **CI-1** : (2 S, 3R)-2,3- dihydro-5-hydroxy-2,3,8,8- tetramethyl-6-(1-phenylethenyl)-4H, 8H-benzo[1,2-b: 3,4-b'] dipyran-4-one (**XIX a**).

(2) **CI-2** : (2R, 3R)-2,3-dihydro-5-hydroxy-2,3,8,8-tetramethyl-6-(1-phenylethynyl)-4H, 8H-benzo [1,2-b: 3,4-b] dipyan-4-one (**XIXc**).

Stigmasterol (**CI-4**) was also isolated for the first time from the leaves of *C. inophyllum*. The following constituents were also isolated confirming the earlier report.

(1) **CI-3 : β-sitosterol**

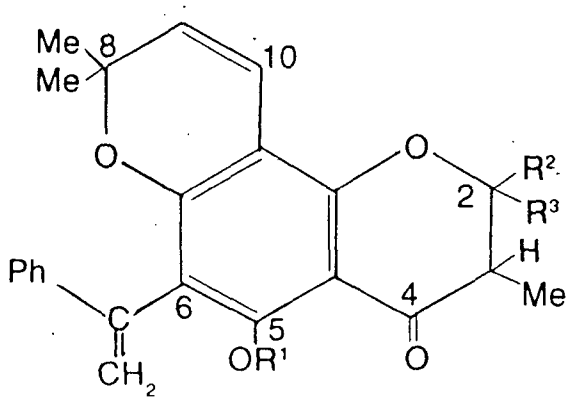
(2) **CI-6 : canophyllol**

(3) **CI-7 : friedelin**

(4) **CI-8 : calaustralin**

(5) **CI-9 : canophyllic acid**

The leaves extracts of *Murraya koenigii* (Rutaceae) were obtained for antifilarial study and simultaneous chemical examination. The following constituents were isolated and characterized by chemical and spectral methods (UV, IR, MS, ^{1}H NMR and ^{13}C NMR).



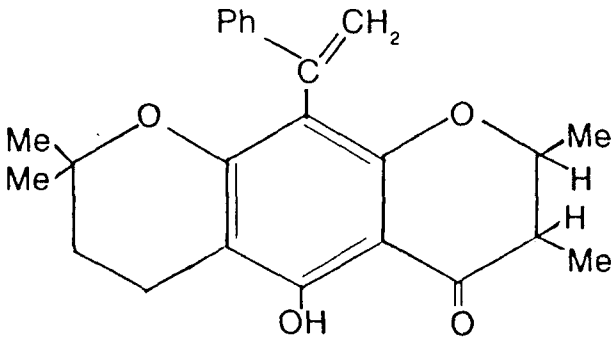
XIX

a $R^1 = R^2 = H$, $R^3 = Me$

b $R^1 = Ac$, $R^2 = H$, $R^3 = Me$

c $R^1 = R^3 = H$, $R^2 = Me$

d $R^1 = Ac$, $R^2 = Me$, $R^3 = H$



XX

(1) **MK-1 :koenimbine = koenimbin**

(2) **MK-2 :β-sitosterol**

(3) **MK-3 :long chain hydrocarbon**

(4) **MK-4 :mahanimbidine = currayangine = murrazoline**

(5) **MK-5 :koenidine = koenigicine = koenimbidine**

(6) **MK-6 :sitosterol-3-O-β-D-glucoside**

The occurrence of b-sitosterol and sitosterol-3-O-β-D-glucoside from *M.koenigii* has not been reported earlier. Although koenimbine and koenimbidine were reported earlier but their ^{13}C NMR and 2D NMR studies have been discussed here to confirm the earlier report.

The roots extracts of *Gerbera lanuginosa* (Compositae) were also obtained for antifilarial study and chemical examination.The following constituents were isolated and characterized by chemical and spectral methods (UV, IR, MS, ^{1}H NMR, ^{13}C NMR).

(1) **GL-1** :mixture of **β-sitosterol** and **stigmasterol**

(2) **GL-2 :hopane** type structure

(3) **GL-3 :taraxerol**

(4) **GL-4 :triacontanoic acid**

(5) **GL-5 :Δ^5 and $\Delta^{5,22}$ sterol-β-D-glucoside**

The occurrence of triacontanoic acid, β-sitosterol, stigmasterol and $\Delta^{5,22}$ sterol-β-D-glucoside from *G. lanuginosa* has not been reported earlier while hopane type triterpene isolated for the first time from the leaves of *G. lanuginosa.*

The preliminary antifilarial screening was made for the extracts of *Asparagus adscendens, Rhododendron arboreum, Rumex hastatus, Gerbera lanuginosa, Mallotus phillippensis, Murraya koenigii, Sencio nudicaulis, Saxifraga stracheyi* and *Ajuga parviflora.* The aqueous and alcoholic extracts of the leaves of *Mallotus philippensis, Sencio nudicaulis* and roots of *Asparagus adscendens, Saxifraga stracheyi* were found both macrofilaricidal (whole worm and n.m. preparation) and microfilaricidal against filarial parasite *Setaria cervi* (Nematoda : Filarioidea) *in vitro.*

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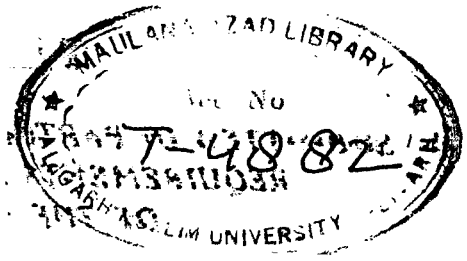

Dedicated

To My

Loving Parents

Dr. Nizam U. Khan
M.Phil., Ph.D.
Reader

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CERTIFICATE

This is to certify that the thesis entitled "**PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF INDIAN MEDICINAL PLANTS**" is the original work carried out by **Mr. Rajinder Singh** under my supervision and is suitable for submission for the partial fulfilment of the requirements for the award of Ph.D. degree in Chemistry.

Nizam U. Khan

Dr. Nizam U. Khan

ACKNOWLEDGMENT

It is good fortune and a matter of privilege for me to have the esteemed supervision of **Dr. Nizam U. Khan** (Reader) for his never failing inspiration, scholarly guidance, constructive criticism, encouraging suggestions in the interpretation of results and above all, sympathy and benevolent attitude.

I am deeply indebted to **Prof. K.C. Singhal** (M.D., Ph.D., FIAN, FIAMS), Chairman, Department of Pharmacology, an eminent scientist, for providing necessary research facilities and to the devotion of much of his precious time to guide me. I can hardly find anyword to express my deepest sense of gratitude to him.

I am thankful to **Prof.N. Islam**, Chiarman, Department of Chemistry, for extending various departmental facilities during the period of study. A large debt of gratitude is owed to my senior colleague, **Dr. (Ms.) Nazneen Parveen** for constructive discussions and encouragement during this work.

Deep appreciation is extended to **Dr. P.B. Singh** (Taxonomist), Regional Research Centre (Ayurvedic), Mandi (H.P.) for collection and identification of plant material.Also, I do not want to waste the oppertunity of acknowledging my gratitude to **Mr. Subhash Varshney** for word processing in time.

I am deeply beholden to my dear **Papa, Mummy** and all other well wishers whose love, support and encouragement are the base of my every success. Lastly but not least, I am extremely thankful to mywife **Mrs. Archana** for her sustained help and buoyancy. She has been a beacon all along to enable me to present this thesis.

Rajinder Singh

(Rajinder Singh)

ABBREVIATION

Ach	acetylcholine
Ac_2O	acetic anhydride
A.P.	Andhra Pradesh
Ar	aryl
$CaCl_2$	calcium chloride
CD	circular dichroism
$CDCl_3$	chloroform
C_5D_5N	pyridine
CHCOSY	heteronucleur shift correlation spectroscopy
cm	centimeter
COLOC	correlation spectroscopy via long range couplings
DEC	diethylcarbamazine
DEPT	distortion less enhancement by polarization transfer
DMAP	4-dimethylaminopyridine
g	gram
µg	microgram
GABA	γ - aminobutyric acid
h	hour (s)
HBMC	heteronuclear multiple quantum correlation
HHCOSY	homonuclear correlation spectroscopy
HIV	human immuno deficiency virus
5-HT	5-hydroxytryptamine
IC	inhibitory concentration
IR	infrared
J&K	Jammu & Kashmir
KCl	potassium chloride
kg	kilogram
l	litre
LC	lethal concentration
µM	micromolar
MEL W.	melarsonil potassium
Mf.	microfilariae
mg	milligram
MHz	megahertz
min	minute (s)
ml	millilitre
M.P.	Madhya Pradesh
m.p.	melting point
MS	mass spectrum
m/z	mass per unit charge
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
ng	nanogram
nM	nanomolar
n.m.	*nurve muscle*
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
PTLC	preparative thin layer chromatography
Rf	retarding force
TLC	thin layer chromatography
UV	ultravoiled
v/v	volume per volume
w	wash
υ	frequency

CONTENTS

CHAPTER - I

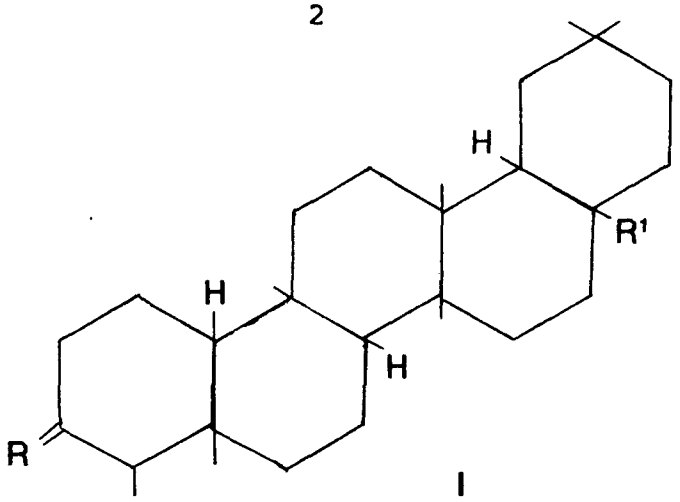
THE CONSTITUENTS OF THE LEAVES OF *CALOPHYLLUM INOPHYLLUM* (GUTTIFEREAE).

INTRODUCTION

TERPENOIDS : Friedelin (**Ia**)[1-3],canophyllol (**Ib**)[1,3], canophyllal (**Ic**)[1], canophyllic acid (**Id**)[1], friedelin-3β-ol (**Ie**)[2], β-sitosterol (**II**)[2] and γ-sitosterol[4] were isolated from leaves and heart wood of *C. inophyllum*. The timber of *C. inophyllum* also contain β-amyrin (**III**)[2].

XANTHONES : 1,5-dihydroxy xanthone (**IVa**)[5], 1,7-dihydroxyxanthone= euxanthone (**IVb**)[6], 1,5,6-trihydroxyxanthone = mesuaxanthone B (**IVc**)[6,7], 1,6-dihydroxy-5 methoxy xanthone = buchanoxanthone (**IVd**)[6], 1,7-dihydro-3,6-dimethoxy xanthone (IVe)[2] were isolated from the heartwood of *C. inophyllum* and 1,3,5,6 - tetrahydroxy-7-methoxy xanthone = caloxanthone E (**IVf**)[8], 1,3,8-trihydroxy-7-methoxy xanthone (**IVg**)[8], 1,3-dihydroxy-7,8-dimethoxy xanthone (**IVh**)[8], 1,3,5-trihydroxy-2-methoxy xanthone (IVi)[8] were isolated from the root bark of *C. inophyllum*. 6-Desoxyjacareubin (**Va**)[2,6,9] and jacareubin (**Vb**)[2,6,7,9] isolated from heartwood of *C. inophyllum* also shows antibacterial activity[10]. Prenylated xanthones isolated from heartwood of *C. inophyllum* were 2-[3,3-dimethyl allyl]-1,3,5-trihydroxy- (**VIa**)[6], -1,3,5,6-tetrahydroxy- (**VIb**)[6,9], -1-hydroxy-3,5,6-trimethoxy- (**VIc**)[6,7], -1-hydroxy-3,5-dimethoxy- (**VId**)[6] xanthone and calophyllin B = 6-(3,3-dimethyl allyl)-1, 5-dihydroxy xanthone (VII)[2,6,7]. Other xanthones isolated from root bark included caloxanthone -A[5], -B[5], -C[11], - D(**VIII**)[8] and macluraxanthone[5].

COUMARINS : Ripe seeds of *C. inophyllum* constitute coumarin calophyllolide (**IX**)[12-16] which shows depressive action on central nervous system[17]. A fungicidal agent tomentolide B(**X**)[18], calaustralin = 5',6' - dimethyl-4'-pyrano-(2',3':6,7)-5-hydroxy-5-prenyl-4-phenyl (**XI**)[19] and ponnolide (**XII**)[13] were also



I

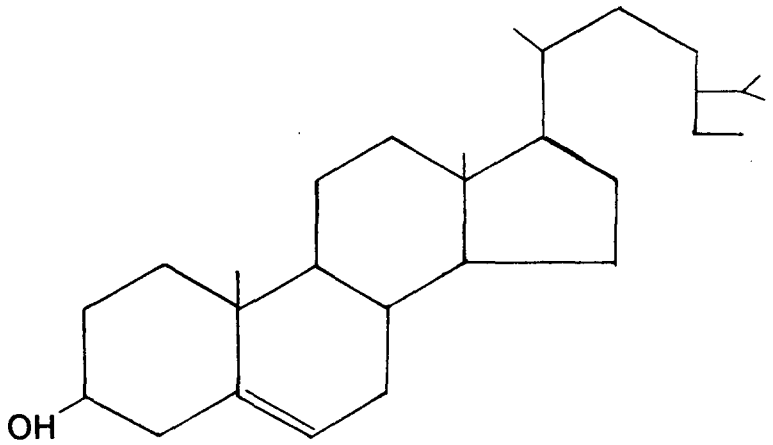
a R = O; $R^1 = CH_3$

b R = O; $R^1 = CH_2OH$

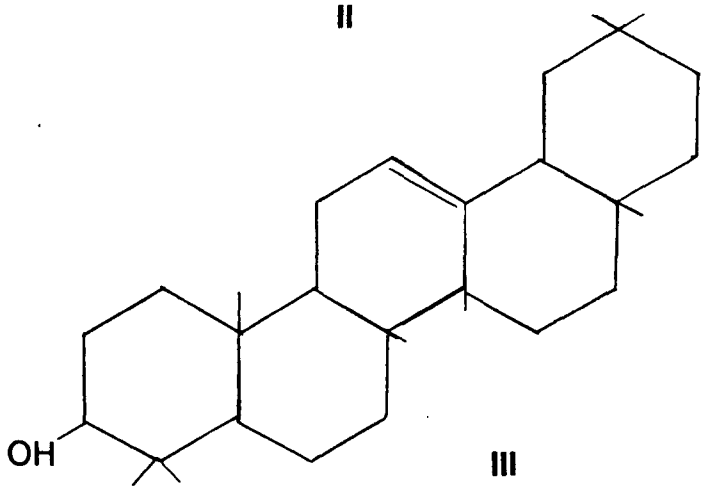
c R = O; $R^1 = CHO$

d R = H, OH; $R^1 = CO_2H$

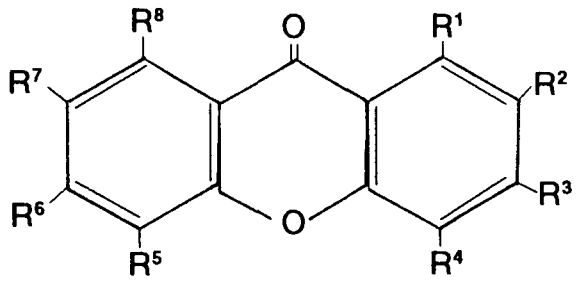
e R = H, OH; $R^1 = CH_3$



II



III



IV

a $R^2 = R^3 = R^4 = R^6 = R^7 = R^8 = H$, $R^1 = R^5 = OH$

b $R^2 = R^3 = R^4 = R^5 = R^6 = R^8 = H$, $R^1 = R^7 = OH$

c $R^2 = R^3 = R^4 = R^7 = R^8 = H$, $R^1 = R^5 = R^6 = OH$

d $R^2 = R^3 = R^4 = R^7 = R^8 = H$, $R^1 = R^6 = OH$, $R = OCH_3$

e $R^2 = R^4 = R^6 = R^8 = H$, $R^1 = R^7 = OH$, $R^3 = R^5 = OCH_3$

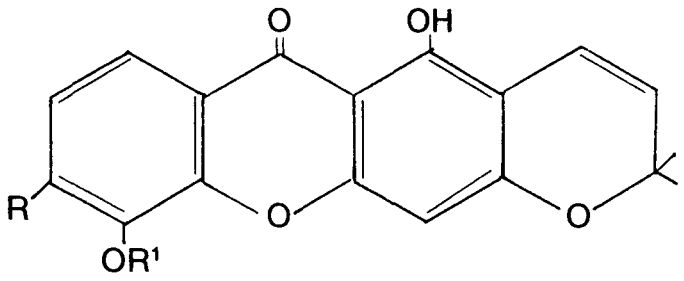
f $R^2 = R^4 = R^8 = H$, $R^1 = R^3 = R^5 = R^6 = OH$, $R^7 = OCH_3$

g $R^2 = R^4 = R^5 = R^6 = H$, $R^1 = R^3 = R^8 = OH$, $R^7 = OCH_3$

h $R^2 = R^4 = R^5 = R^6 = H$, $R^1 = R^3 = OH$, $R^7 = R^8 = OCH_3$

i $R^4 = R^6 = R^7 = R^8 = H$, $R^1 = R^3 = R^5 = OH$, $R^2 = OCH_3$

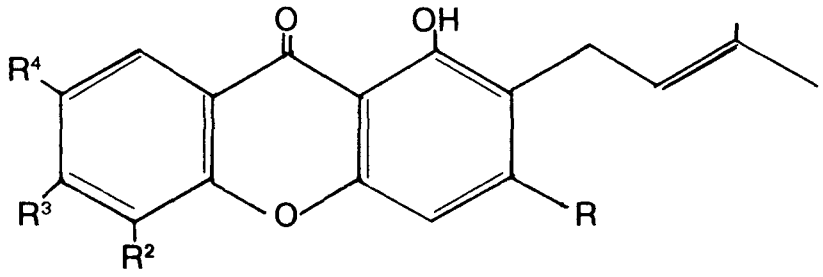
j $R^2 = R^3 = R^4 = R^7 = R^8 = H$, $R^6 = OH$, $R^1 = R^5 = OCH_3$



V

a $R = R^1 = H$

b $R = OH$, $R^1 = H$



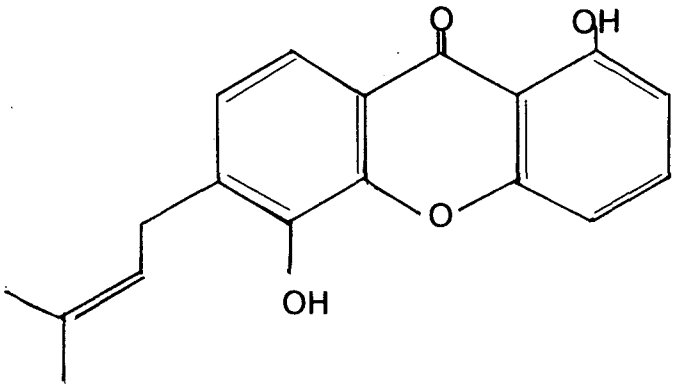
VI

a $R^3 = R^4 = H$, $R^1 = R^2 = OH$

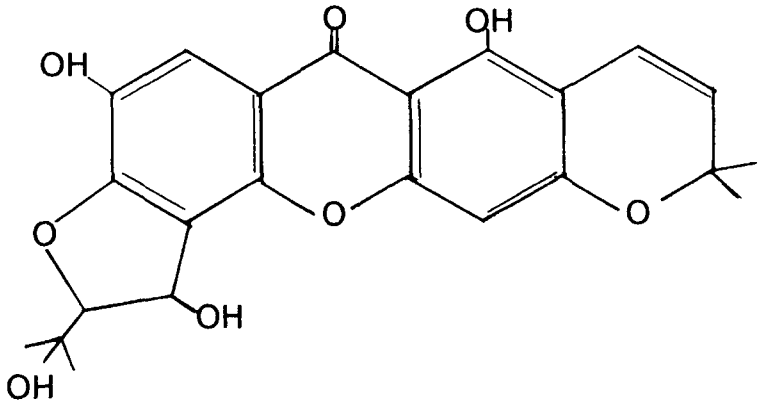
b $R^4 = H$, $R^1 = R^2 = R^3 = OH$

c $R^4 = H$, $R^1 = R^2 = R^3 = OCH_3$

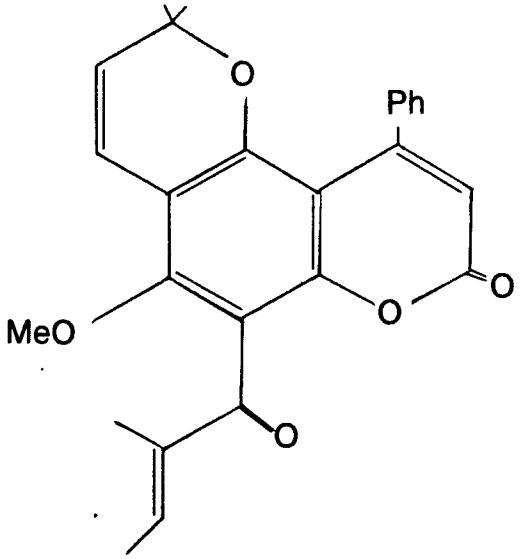
d $R^3 = R^4 = H$, $R^1 = R^2 = OCH_3$



VII



VIII



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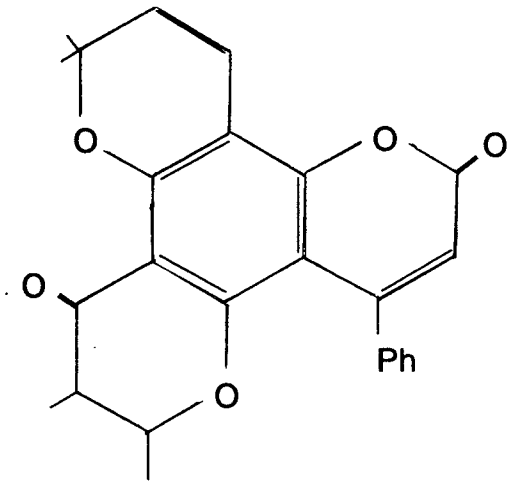
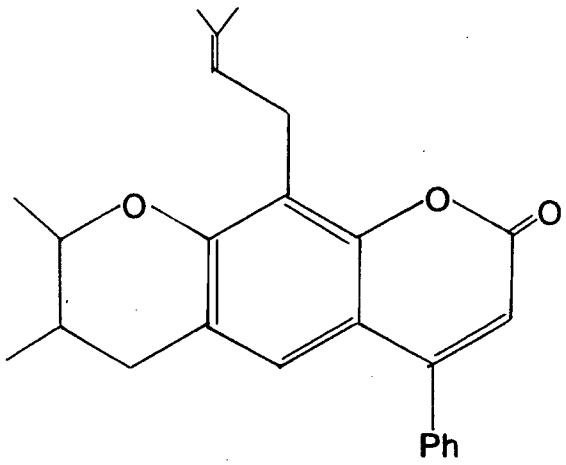
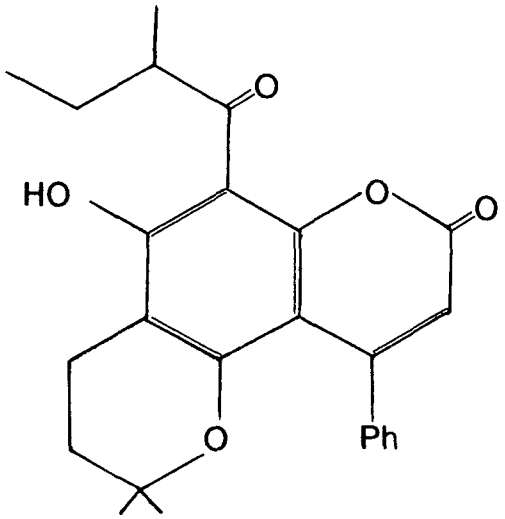


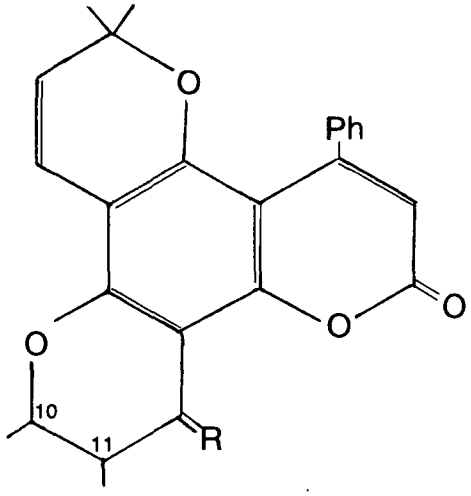
X



XI



XII



XIII

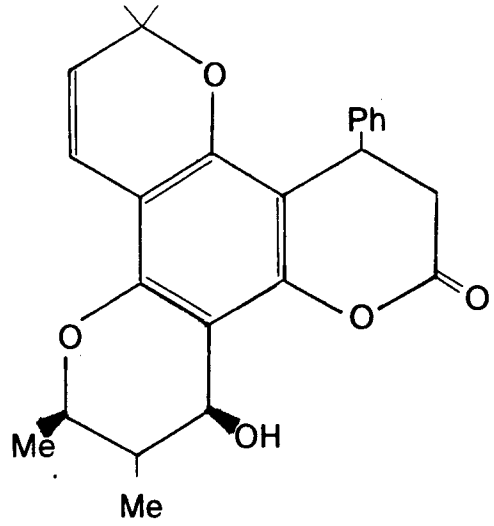
a R = H, OH ; 10, 11 - cis

b R = H, OH ; 10, 11 - trans

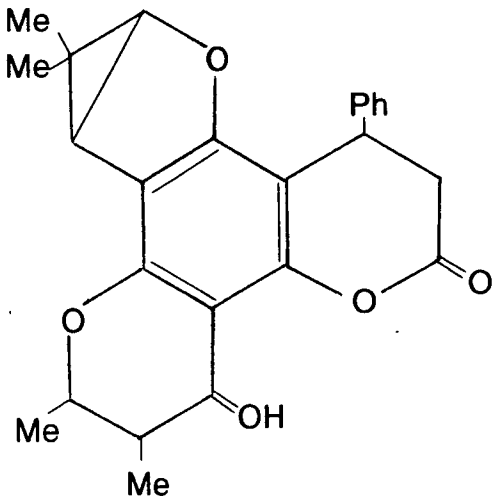
c R = O ; 10, 11 - trans

d R = O, OH ; 10, 11 - cis

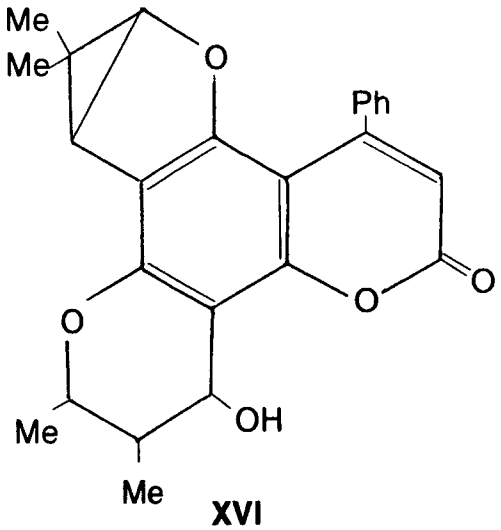
e R = O ; 10, 11 - cis



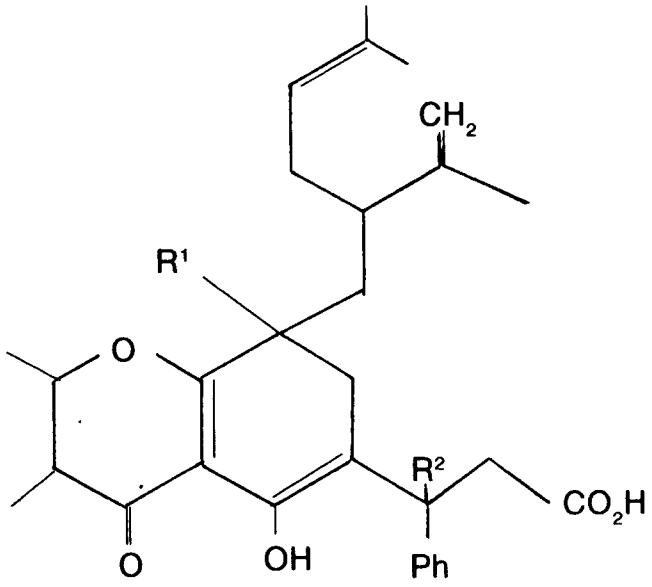
XIV



XV



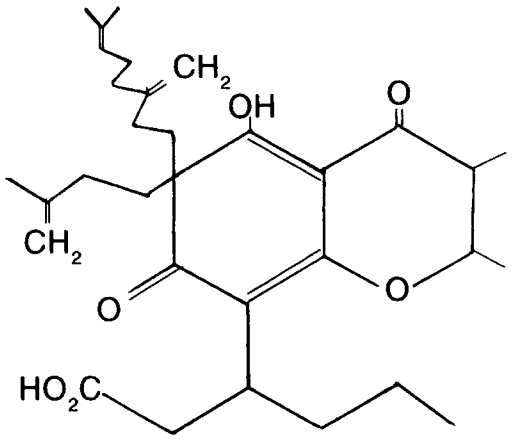
XVI



XVII

a $R^1 = CH_3$, $R^2 = CH_3$

b $R^1 = CH_2CH{:}CMe_2$, $R^2 = H$



XVIII

isolated from the seeds of *C. inophyllum*, Inophyllum-A (**XIIIa**)[16,20,21] B(**XIIIb**)[16,20,21], -C = trans inophylloide (**XIIIc**)[12,13,16,20,21], -D(**XIIId**)[16,20,21], - E = cis inophylloide (**XIIIe**)[12.13,16,20,21] isolated from leaves and ripe seeds of *C. inophyllum* shows piscidal activity[21]. Inophyllums P (**XIV**)[16], G-1 (**XV**)[17] and G-3 (**XVI**)[16] were also isolated from the leaves of *C. inophyllum*, Inophyllum B and P inhibited HIV reverse transcriptase with IC_{50} values of 38 and 130 nM respectively and both were active against HIV-1 in cell culture[16] (IC_{50} of 1.4 and 1.6 μM).

FLAVONOIDS AND NEOFLAVONOIDS : Flavonoids amentoflavone[3], pyranoamentoflavone[3], myricetin[22], quercetin[22], myricetin-7-glucoside[22,23] were isolated from heartwood and androceum of flowers. Two neoflavonoids (+) -(2R,3S)-2,3-dimethyl-5-hydroxy-6-(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenyl ethyl)-2,3-dihydro-benzo pyran[3] and (+) -(2R,3S,16S)-2,3-dimethyl-5-hydroxy-6(3-methylbut-2-enyl)-7-methoxy-8-(2-carboxyl-1-phenyl ethyl)-2,3-dihydrobenzopyranal-2-one[24] were also isolated from heartwood of *C. inophyllum*.

MISCELLANEOUS : Calophyllic acid (**XVIIa**)[13,15], calophynic acid (**XVIIb**)[25], inophyllic acid[13,15] and inophylloidic acid (**XVIII**)[26] along with erythrodiol-3-acetate[4], (-) epicatechin[5], cinnamic acid[5] and leucoanthocyanidin[22] occured in seeds, bark and petals of *C. inophyllum*.

RESULTS AND DISCUSSION

A petrol extract of *C. inophyllum* on column chromatography followed by recrystallization from $CHCl_3$- EtOH afforded a new compound, **CI-1** (**XIXa**) which analysed for $C_{24}H_{24}O_4$ (M^+ m/z 376). The IR spectrum showed a strong band due to chelated aromatic carbonyl group (1625 cm^{-1}), along with peaks at 785 and 700 cm^{-1}, suggesting the presence of a monosubstituted benzene ring[20]. The ^1H NMR spectrum (Table I, Fig. 1) showed a one - proton exchangeable signal at δ12.49 characteristic of a chelated OH group and a five proton multiplet centred at δ7.32 ascribed to the phenyl group. Other signals in the spectrum showed the presence of the following functionalities through decoupling experiments : (i) a dimethyl benzo - γ - pyrone [Ar- O - CH (Me) - CH (Me) - CO - Ar] unit, with one of the methine protons ($J_{2H, 3H}$, $3H_Z$) equatorially oriented, (ii) a 2,2 - dimethyl benzo [b] pyran unit and (iii) a $>C=CH_2$ grouping.

The above data could be fitted with either structure **XIX a** or **XX**. In order to discriminate between the two structures, the compound was acetylated (Ac_2O/ pyridine/DMAP/CH_2Cl_2)[27].Since hydroxy chromones exert a significant shielding influence on peri-protons[28,29], the concerned proton of the pyrone ring should have been shielded in the acetate if the structures were **XX**. Actually, the pyran protons were marginally (0.03-0.05 ppm) deshielded, while the methylene protons of $>CH=CH_2$ appeared shielded by 0.13 ppm and 0.07 ppm, respectively, for the lowest field and higher signals. Structure **XIXa** and **XIXb** are thus preferred for the compound and its acetate, respectively.

In the mass spectrum, the $[M]^+$ appeared at m/z 376, the base peak being at m/z 361 for the loss of a methyl group. The other prominent peak was at

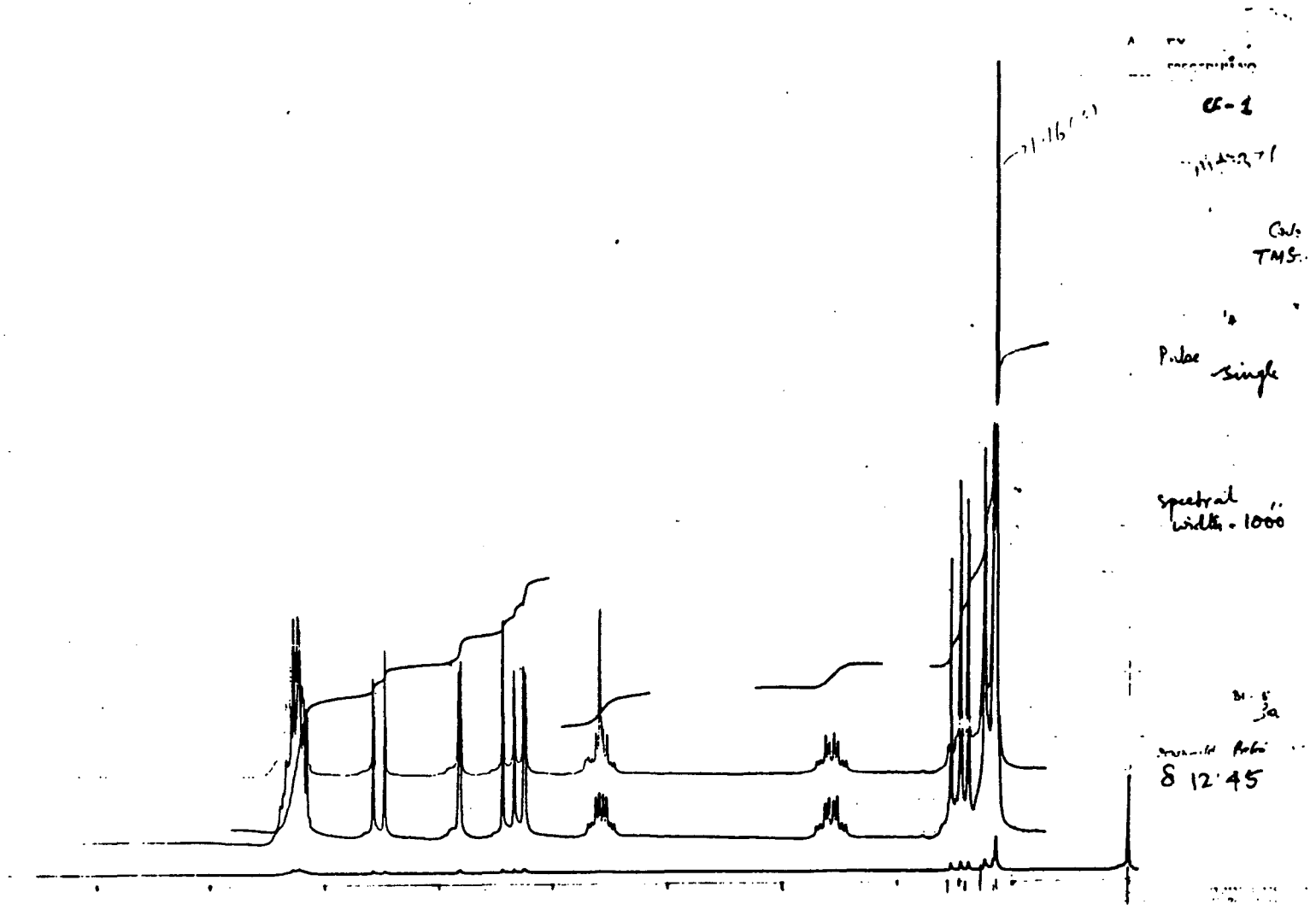
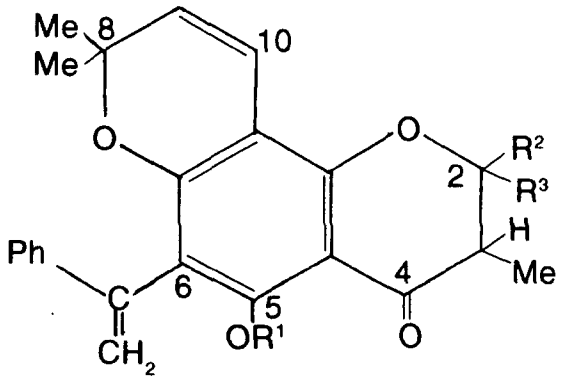


Fig.1



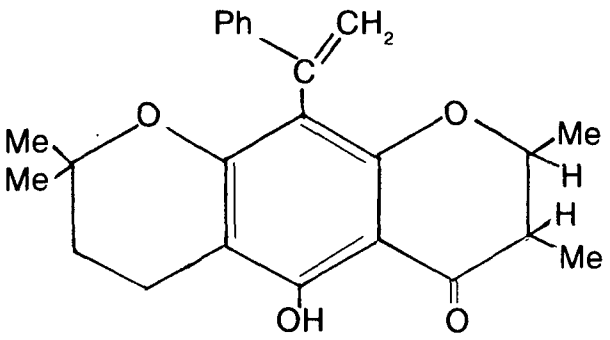
XIX

a $R^1 = R^2 = H$, $R^3 = Me$

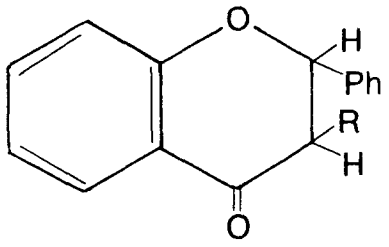
b $R^1 = Ac$, $R^2 = H$, $R^3 = Me$

c $R^1 = R^3 = H$, $R^2 = Me$

d $R^1 = Ac$, $R^2 = Me$, $R^3 = H$



XX



XXI

a R = H

b R = OH

m/z 305 which originated from the $[M-Me]^+$ peak by loss of 56 m.u. (Calc.m* 257.6; observed around 258), conceivably via retro-Diels Alder cleavage of the dimethyl benzo pyrone ring with expulsion of the C_4H_8 unit. Thus, compound **CI-1** was characterized as (2S, 3R) 2,3-dihydro-5-hydroxy-2,3,8,8-tetramethyl-6-(1-phenylethenyl)-4H, 8H-benzo [1,2-b:3,4-b'] dipyran-4-one *(**XIXa**)*.

Table I. ^{1}H NMR data of compounds CI-1 and CI-2 (100 MHz, $CDCl_3$)

Assignment	Chemical shifts (δ, ppm) for	
	CI-1 (XIXa)	**CI-2 (XIXc)**
8-Me_2	1.16 (s)	1.12 (s)
		1.16 (s)
3-Me	1.22 (d, J=7Hz)	1.16 (s),1.22 (d, J=7Hz)
2-Me	1.44 (d, J=7Hz)	1.54 (d,J=6.5 Hz)
3-H	2.60 (dq, J=7, 3Hz)	2.60 (dq, J=11, 7Hz)
2-H	4.63(dq,J=7,3Hz)	4.25 (dq,J=11, 6.5 Hz)
9-H	5.46 (d, J=10Hz)	5.45(d,J=10Hz)
10-H	6.58 (d,J=10Hz)	6.57(d,J=10Hz)
=CH2	5.30 (d,J=2Hz)	5.29(d,J=2Hz)
	5.88 (d, J=2Hz)	5.85 (d, J=2Hz)
-Ph	7.32(m)	7.30(m)
-OH	12.49 (s)	12.56 (s)

Compound **CI-2** was recrystallized from $CHCl_3$-EtOH.The molecular for-

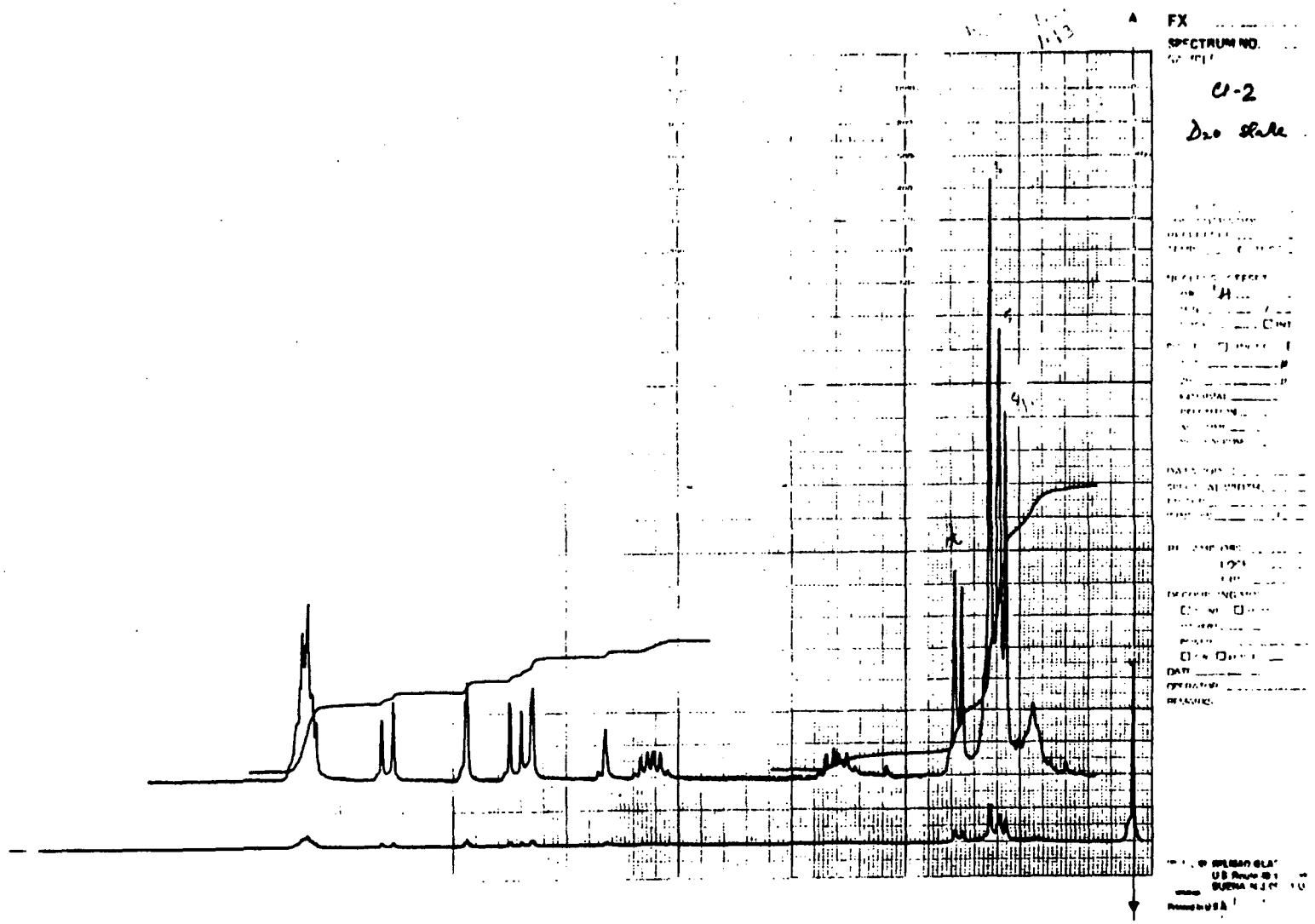
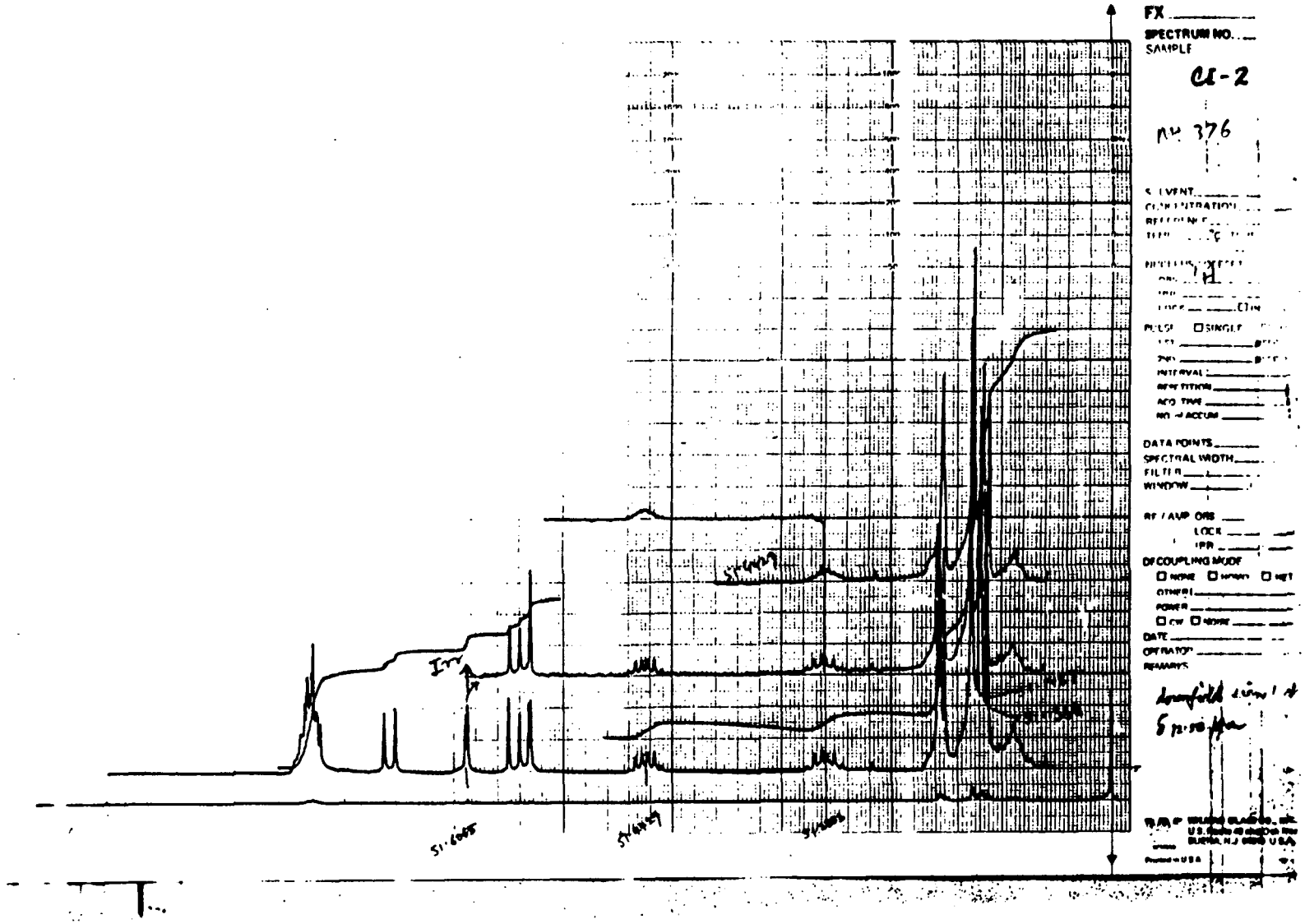


Fig.2a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DATE
OPERATOR
REMARKS

Fig.2b

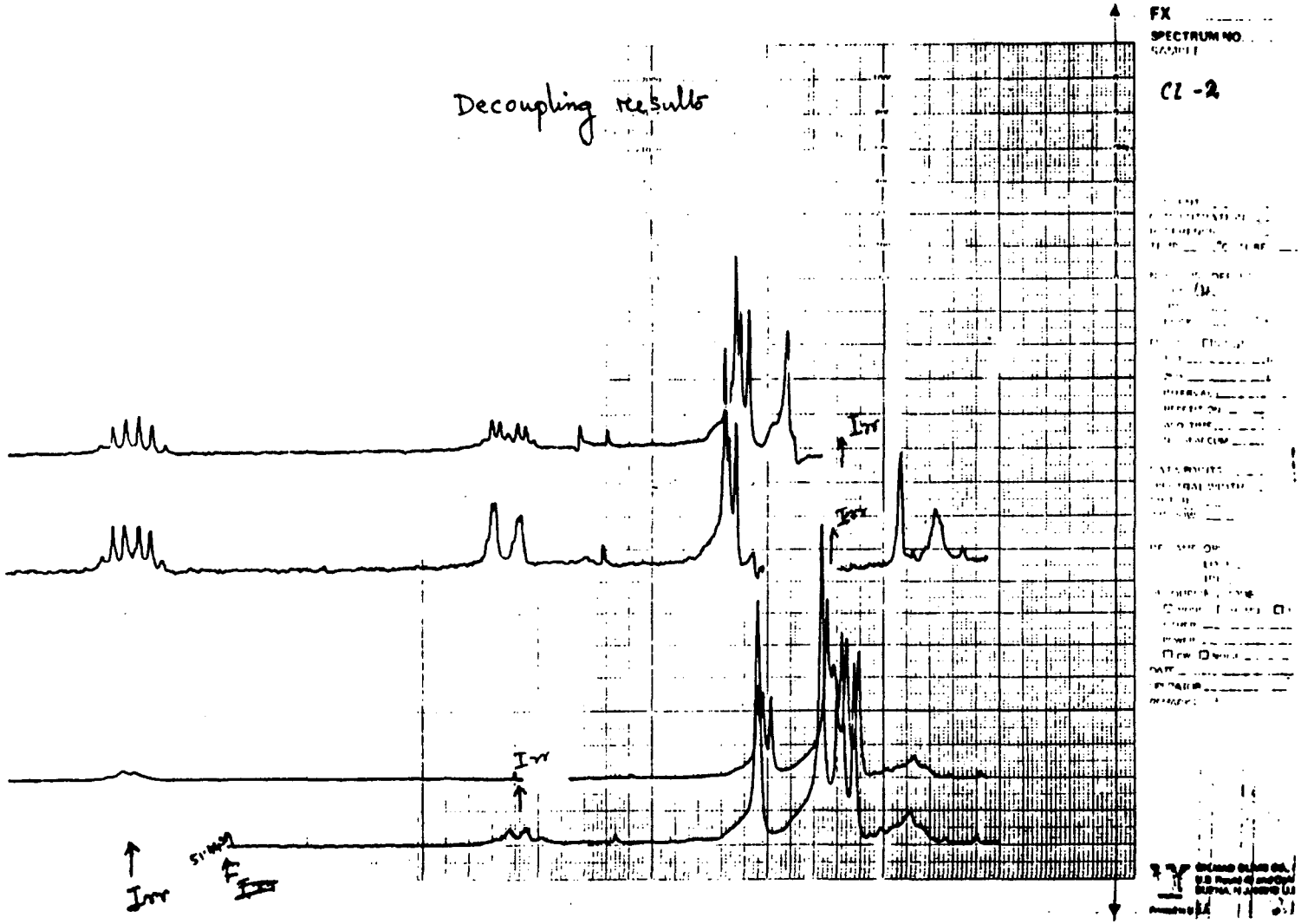

Fig.3

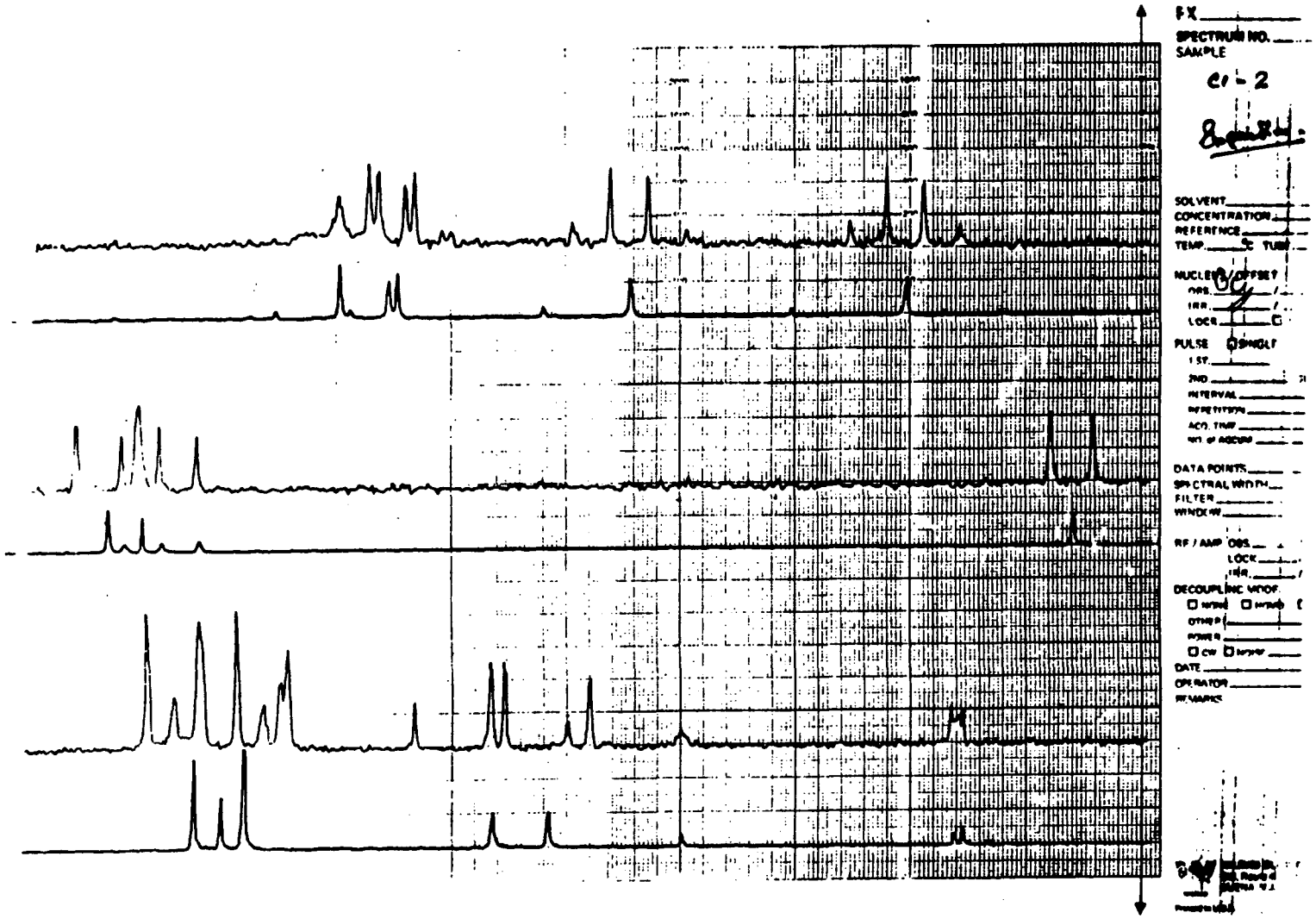
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Fig.4

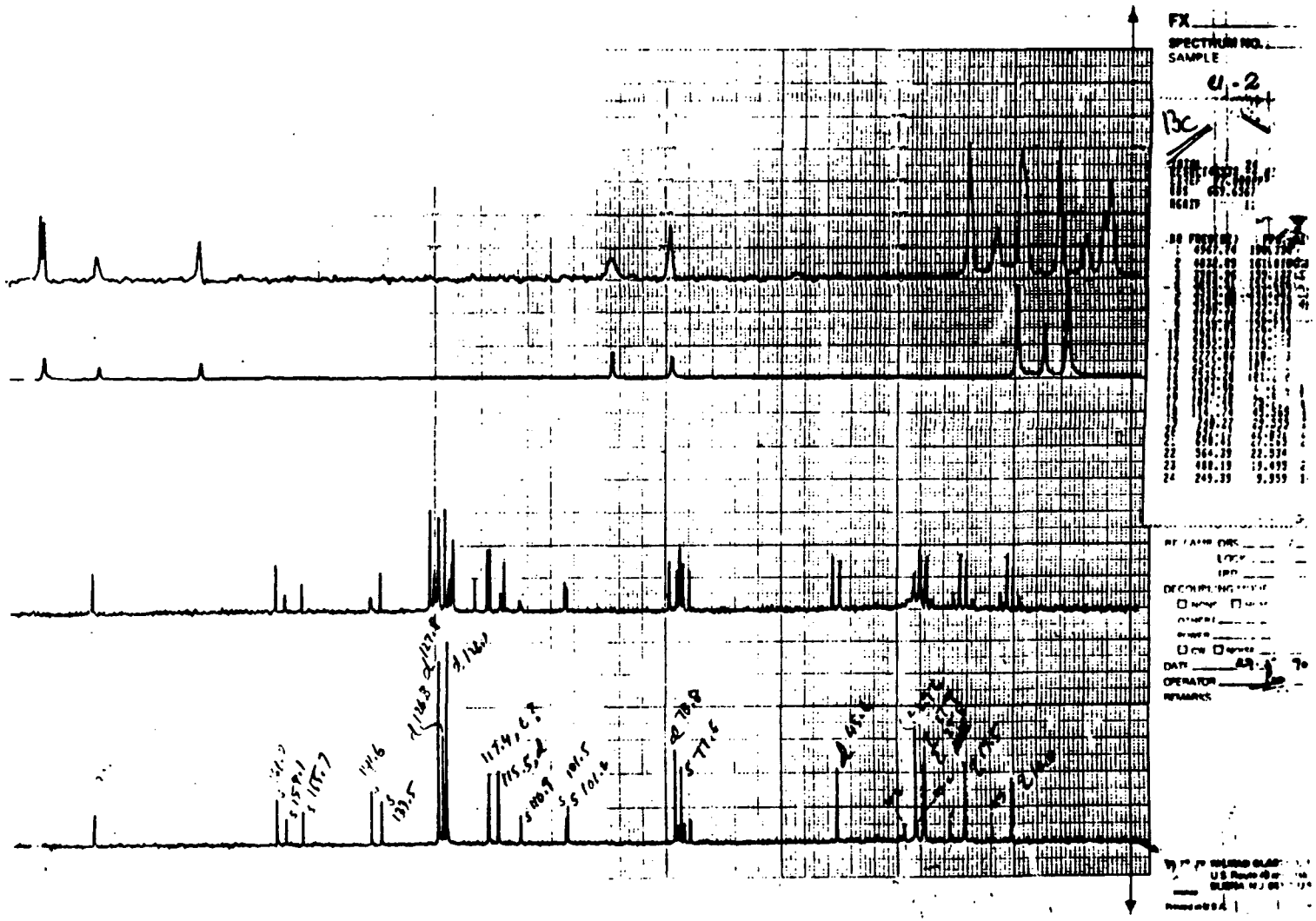


Fig.5

mula $C_{24}H_{24}O_4$, was assigned to it from the mass spectrum ($[M]^+$ m/z 376), which was very similar to that of **CI-1**, showing their isomeric nature. Most of the ^1H NMR (Table 1, Fig 2a & 2b) signals, including acetylation shifts (shielding of $>C=CH_2$ protons in **XIXd** by 0.14 and 0.07 ppm) were also identical . However, the resonance position of 2-H of the pyrone ring (δ4.25, J=6.5 Hz) appeared shifted upfield by 0.38 ppm and the $J_{2H, 3H}$ value was also much higher . A decoupling experiment (Fig. 3 & 4) showed the presence of a dimethyl benzo - γ - pyrone [Ar - O -CH (Me) - CH (Me) - CO - Ar] unit with the two methine protons trans - diaxially oriented, a 2, 2 - dimethyl benzo [b] pyran unit and a $>C=CH_2$ grouping.

The ^{13}C NMR spectrum (Fig. 5) of **CI-2** showed signals for all the 24 carbon atoms. The two methine signals at δ 115.5 and 126.1 , together with methyl signals at δ 27.6 and 27.9, and also signals at δ 77.6 (O-C—) agreed well with the presence of a 2,2- dimethyl benzo [b] pyran ring system [30]. The six singlets in the downfield region at δ 161.0, 159.1 , 155.7, 110.9, 101.5 and 101.2 , in conjuction with the carbonyl signals at δ 198.3 suggested the presence of a 5- hydroxy - 7 - oxy - 6, 8 - disubstituted - benzo - γ - pyrone moiety [31]. Signals at δ 78.8 (O-CH), 45.6 (CO-CH), 19.5 (CH_3-CH-O-) and 10.0 (CH_3-CH-CO) were in agreement with the presence of 2, 3 - dimethyl substitution in the pyrone ring. The remaining peaks at δ 139.5 (s) and 117.4 (t, $>C=CH_2$) along with those at δ 126.1, 127.8 (each accounting for two methine carbons), 126.8 (CH) and 141.6 (–C–) confirmed the presence of the Ph-C=CH_2 unit. These data confirm that the compound **CI-2** has structure **XIXc** which was found to be the isomer of **CI-1** (**XIXa**). Since the completion of our work, the structure of **CI-2** isolated from another plant *C. tomentosum* has been reported [32]. Our data are in good agreement with those published by Babu et al [32], except that the

previously assigned chemical shifts for 3-H and 2-H (and those of the substituent methyl groups) need to be reversed. The ^{13}C NMR of **CI-2** was not given earlier. Also the absolute configuration was not determined earlier.

Calaustralin (**XI**) m.p. 182 (lit. 190-91°C) was also isolated from chloroform fraction of *C. inophyllum* (labelled as **CI-8**)for stereochemical studies. The mass and ^1H NMR spectra (Table -II) of **XI** indicated its identity with calaustralin isolated earlier from the same plant [19].

Table II. ^1H NMR data of CI-8 (XI) (100 MHz, $CDCl_3$)

Assignment	Chemical Shift (δ, ppm)
3'- Me_2	1.70 (s)
	1.86 (s)
7-Me	1.24 (d, J=7)
8-Me	1.58 (d, J=7)
7-H	2.66 (dq, J=11,7)
8-H	4.30 (dq, J=11,7)
2'-H	5.28 (t, J=8)
3-H	6.02 (s)
1'-H_2	3.49 (d, J=8)
Ph	7.40 (m)
–OH	13.14 (s)

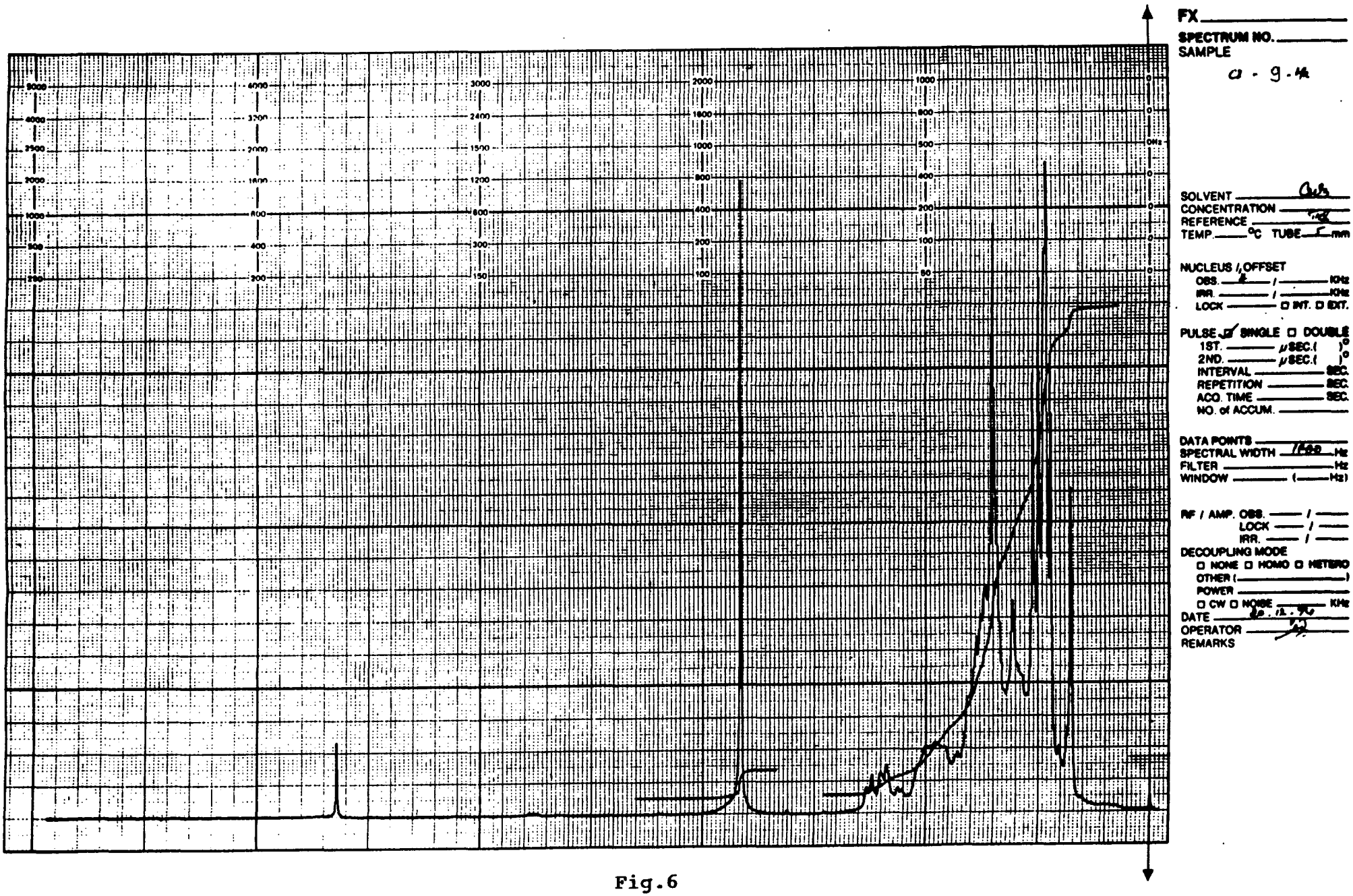
The application of chiroptical methods for the assignment of absolute

configuration of such dimethyl bezo - γ - pyrones does not appear to have been well documented. However, CD has been used in the case of related flavones or even 3 - substituted flavanones. Opposite Cotton effects have been observed for the n - π^* transition around 320 nm and π - π^* transition around 290 nm of the arylcarbonyl chromophore, the relative signs depending on the chirality of the conjugated chromophore [33,34]. Thus, both the flavanones **XXIa** (2R) and 3 - substituted flavanone **XXIb** (2S, 3S) with "equivalent C-2 configuration" showed a negative maximum around 330 nm and a positive one around 280 - 290 nm. We therefore, applied this method to **XIXa**, **XIXc** and **XI**. The CD curve of the compounds showed a positive Cotton effect around 315 nm and a negative one at around 292 nm [CD (MeOH) **CI-1**: $292^{-3.61}$, $315^{+1.57}$; **CI-2** : $292^{-3.39}$, $315^{+0.64}$]. Therefore , the absolute configuration of **CI-2 (XIXc)** and **CI-8** (**XI**) should be 2R, 3R. As **CI-2** differ from **CI-1** only in the configuration of C-2, its absolute configuration has been fixed as 2S, 3R.

The chloroform extract of *C. inophyllum* on column chromatography, preparative thin layer chromatography (PTLC) and crystallization yielded compounds lebelled as **CI-3**, **CI-4** , **CI-5**, **CI-6**, **CI-7**, **CI-8** and **CI-9** .

CI-3 was obtained as colourless crystals by elution of the column with petrol - benzene (1:1) . Its m.p., Rf, IR, MS and ^{1}H NMR sepctral data were comparable with the authentic sample of β-sitosterol. Therefore, the earlier report[2] of ***β - sitosterol*** **(II)** from the leaves of *C. inophyllum* is further confirmed.

CI-4 was obtained as colourless, crystalline compound on further elution of the column with petrol - benzene (1:1). The chemical and spectral data of this fraction indicated the presence of ***stigmasterol*** (not reported from this plant earlier) contaminated with ***β-sitosterol***.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CONCENTRATION
REFERENCE
TEMP. °C TUBE mm
NUCLEUS / OFFSET
OBS. / KHz
IRR. / KHz
LOCK ☐ INT. ☐ EXT.
PULSE ☑ SINGLE ☐ DOUBLE
1ST. μSEC.( )°
2ND. μSEC.( )°
INTERVAL SEC.
REPETITION SEC.
ACQ. TIME SEC.
NO. of ACCUM.
DATA POINTS
SPECTRAL WIDTH 1500 Hz
FILTER Hz
WINDOW (Hz)
RF / AMP. OBS. /
LOCK /
IRR. /
DECOUPLING MODE
☐ NONE ☐ HOMO ☐ HETERO
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Fig.6

The elution of the column with petrol - benzene (2:8) followed by recrystallization afforded colourless granules of **CI-5** melting at 88.9°C. From infra red spectrum of **CI-5** it can be inferred that the compound is a **long chain alcohol**.

The fractionation of column with benzene as eluent yielded **CI-6**, **CI-7** and **CI-8** . **CI-6** was obtained as green needle shaped crystals from cholorofrom, melting at 280°C. Its chemical and spectral data (IR, MS and ^{1}H NMR) were comparable with the authentic sample of **canophyllol (Ib)**, thus confirming the earlier report [1,3] of this compound from *C. inophyllum*. The TLC examination of **CI-7** on silica gel showed it to be impure. It was purified by PTLC and crystallized from chloroform - ethanol as colourless crystalline compound, melting at 267°C. The chemical and spectral data (IR and ^{1}H NMR) of **CI-7** compared well with the authentic sample of **friedelin (Ia)** confirming its earlier report [1,3]. **CI-8** was obtained as light yellow crystals from chloroform. Its chemical and spectral data (IR, MS and ^{1}H NMR) were comparable with the authentic sample of **calaustralin (XI)** isolated from this plant earlier[19]. ^{1}H NMR spectrum of **CI-8** is given in Table II. However, **CI-8** was subjected to chiroptical studies, and the absolute configuration of **CI-8** was assigned as [2R, 3R] by comparison of ^{1}H NMR (Table -I, -II) and stereochemistry with **CI-2**.

The elution of column with benzene - ethyl acetate (1:1) yielded **CI-9** which was crystallized from chloroform - ethanol as colourless compound, melting at 306-8°C . From chemical and IR spectral data the compound was indicative of a carboxylic acid. It gave a methyl ester melting at 215-17°C on esterification. From IR and ^{1}H NMR (Fig. 6) of methyl ester of **CI-9** it was characterized as **canophyllic acid (Id)**.

EXPERIMENTAL

Air dried and powdered leaves (1.7 kg) of *C. inophyllum* were extracted with ethanol. Repeated extractions were made until the solution became colourless. The ethanol extract was evaporated under reduced pressure. The dark green gummy mass (80 g) so obtained was treated with petrol (60-80°), chloroform and methanol.

A. Compounds isolated from petrol fraction :

The petrol extract was concentrated under vacuum. The crude product (25 g) was adsorbed on silica gel and chromatographed on silica gel column. Elution of column with petrol-benzene (4:1) afforded **CI-1** which was further crystallized from chloroform-ethanol.

CI-1 : Needle shaped crystals (35 mg), soluble in chloroform and melt at 167°C. Rf= 0.35 (silica gel, petrol-acetone, 93:7). $[\alpha]_D$ + 29.5° (c0.52, $CHCl_3$).

Spectral data of CI-1 (XIXa).

UV λ_{max}^{MeOH} nm (log ε) : 232 (4.32), 265 (4.56), 273 (4.56), 299 (3.99), 310 (3.95), 365 (3.49).

IR υ_{max}^{KBr} cm^{-1} : 3400, 2950, 1645, 1625, 1595, 1460, 1445, 1418, 1392, 1350, 1315,1292, 1262, 1245, 1195, 1130, 1120, 1028, 980, 915, 830, 785, 750, 700.

MS m/z (rel. int.) : 376$[M]^+$ (45), 361[$[M-Me]^+$ (100), 305 $[M-Me-C_4H_8]^+$ (89), 277 $[M-Me-C_4H_8-CO]^+$ (8).

^{1}H NMR data is given in the discussion (Table I).

Further elution of column with petrol-benzene (4:1) and recrystallization

from chloroform-ethanol gave crystals of **Cl-2**.

Cl-2 : Crystalline compound (40 mg) which dissolve readily in chloroform and melt at 132°C. Rf = 0.43 (silica gel, petrol-acetone, 93:7). $[\alpha]_D$ + 56.1° (c1.39, $CHCl_3$).

Spectral data of Cl-2 (XIXc).

UV λ_{max}^{MeOH} nm(log ε) : 231 (4.41), 265 (4.60), 273 (4.60), 299 (4.04), 309 (3.97),364 (3.50).

IR ν_{max}^{KBr} cm^{-1} : 3400, 2900, 1645, 1625, 1595, 1460, 1445, 1415, 1385, 1360, 1285, 1245, 1190, 1165, 1145, 1130, 1050, 980, 920, 785, 700.

MS m/z (rel. int.) : 376 $[M]^+$, 361 (100), 305 (89), 277 (8), 161 (6), 58 (12).

^{1}H NMR data is given in the discussion (Table - I).

B. Compounds isolated from chloroform fraction :

The chloroform extract was concentrated under vacuum. The crude product (30 g) was adsorbed on silica gel and chromatographed on silica gel column set with petrol (60-80°). Elution of the column with petrol-benzene (1:1) afforded a compound which was crystallized from chloroform-ethanol as colourless crystals of **Cl-3** and **Cl-4**.

Cl-3 : Colourless crystals (60 mg), dissolves readily in chloroform and melt at 142-3°C. Rf=0.73 (silica gel, benzene-acetone, 9:1). UV inactive but appeared on absorption with iodine and charring with perchloric acid.

IR, MS and 1H NMR data of Cl-3.

IR ν_{max}^{KBr} cm^{-1} : 3400-3250 (br), 2900, 2850, 1640, 1370, 1050, 950, 880.

MS m/z (rel. int.) : 414 $[M]^+$ (100), 399 (25), 396 (30), 381 (18), 273 (50), 271 (44), 255 (60), 231 (30), 213 (50).

^{1}H NMR (100 MHz) : δ0.68 (s, 3H, 18-Me), 0.75 (d, J=6.8 Hz,3H, 28-Me), 0.84 (d, J=6.5Hz, 6H, 26, 27-Me), 0.92 (d, J = 6.5 Hz, 3H, 21-Me), 1.01 (s, 3H, 19-Me), 3.52 (m, 1H, Olefinic proton), 1.07-2.34 (-CH_2 and -CH protons of cyclic system and side chain).

From IR, MS, ^{1}H NMR spectral data and direct comparison with authentic sample the compound was found to be **β-sitosterol**.

CI-4 : Colourless crystalline compound (25 mg), soluble in chloroform and melt at 158°C. Rf = 0.76 (silica gel, benzene-acetone, 9:1). UV inactive, appeared on absorption with iodine and charring with perchloric acid.

IR data of CI-4.

IR ν_{max}^{KBr} cm^{-1} : 3400, 3200, 2900, 2850, 1720, 1480, 1460, 1370, 1360, 1240, 1040, 1010, 950.

From IR spectral data the compound was found to be a mixture of **β-sitosterol** and **stigmasterol**.

CI-5 : Further elution of column with petrol-benzene (2:8) and recrystallization with chloroform ethanol afforded colourless granules of **CI-5** (30 mg), m.p. 88-9°C, Rf = 0.60 (silica gel, benzene-chloroform, 9:1). Developed by iodine and perchloric acid.

IR ν_{max}^{KBr} cm^{-1} : 3350-3200(br), 2900, 2850, 1460, 1050, 1010,720.

CI-5 may be an open chain, hydrogen-bonded **alcohol**.

Elution of the column only with benzene and recrystallization with chloroform - ethanol afforded **CI-6**, **CI-7** and **CI-8**.

CI-6 : Light green needle-shaped crystals (48mg), soluble in chloroform, m.p. 280°C. Rf=0.75 (silica gel, benzene-acetone, 9:1). UV inactive, appeared on

absorption of idoine and charring with perchloric acid.

IR, MS and ^{1}H NMR data of CI-6.

IR ν_{max}^{KBr} cm^{-1} : 3550, 2900, 1705, 1460, 1390, 1360, 1115, 1080, 1050, 1000, 910, 885.

MS m/z (rel. int.): 442 $[M]^+$ (1), 411 (90), 301 (10), 296 (11), 273 (49), 247 (16), 137 (100), 110 (80), 95 (89).

^{1}H NMR (100MHz): δ 0.68 (s, 3H,-CH_3), 0.83 (s,3H,-CH_3), 0.91 (s, 6H,2xCH_3), 0.99 (s, 6H, 2 x CH_3), 1.06 (s,3H, CH_3), 1.35-1.84 (m, CH_2 and CH), 2.2-2.4 (m, protons at C-2 and C-4), 3.65 (s,2H, CH_2OH).

From IR, MS and ^{1}H NMR spectral data the compound was found to be **canophyllol**.

CI-7 : It was further purified by applying prepartive thin layer chromatography and recrystallization with chloroform - ethanol to gave colourless crystalline compound (20mg) which dissolved readily in chloroform and melt at 267°C. Rf=0.86 (silica gel, benzene). UV inactive, developed on charring with percholric acid and absorption of iodine.

IR and ^{1}H NMR data of CI-7.

IR ν_{max}^{KBr} cm^{-1} : 2900, 2850, 1715, 1460, 1440, 1390.

^{1}H NMR (100MHz): δ0.68 (s, 3H,-CH_3), 0.83 (s, 3H,-CH_3), 0.85 (s, 3H,-CH_3),0.87 (s, 3H,-CH_3), 0.99 (s, 3H,-CH_3), 1.02 (s, 6H,-Me_2), 1.04 (s, 3H,-CH_3), 1.21-1.60 (-CH_2, - CH), 2.22-2.32 (m, 2-H_2), 2.39 (s, 1H, 4-H)

From IR, ^{1}H NMR and direct comparison with authentic sample **CI-7** was identified as **friedelin**.

CI-8 : Light yellow crystals (58 mg), soluble in chloroform, m.p. 189°C. Rf=0.79 (silica gel, benzene), yellow with naked eye, brown in UV light and gave light green colouration with alcoholic $FeCl_3$.

IR, MS and ^{1}H MNR data of CI-8.

IR ν_{max}^{KBr} cm^{-1} : 3400, 2900, 1730, 1630, 1600, 1560, 1440, 1380, 1345, 1285, 1250, 1230, 1185, 1155, 1110, 1080, 1055, 940, 895, 830, 770, 700.

MS m/z (rel. int.): 404 $[M]^+$ (100), 389 (95), 361 (94), 343 (64), 336 (59.5), 333 (59.5), metastable 332.6 (5.6), 305 (75.3), 297 (37.6), 280 (24.1), 277 (28.8), 202 (17.6).

^{1}H NMR (100 MHz) : δ1.24 (d, J=7 Hz, 7-Me), 1.58 (d, J=7 Hz, 8-Me), 1.70 (s,3'-Me), 1.86 (s, 3'-Me), 2.66 (dq, J=11, 7Hz, 7-H), 3.49 (d, J=8Hz, 1'-H_2), 4.30 (dq, J=11, 7 Hz, 8-H), 5.28 (t, J=8Hz, 2'-H), 6.02 (s, 3-H), 7.40 (m, Ph), 13.14 (s,-0H).

From IR, MS and ^{1}H NMR spectral data the compound was found to be **calaustralin**.

Further elution of column with benzene ethyl acetate (1:1) and on recrystalization from chloroform-ethanol **CI-9** was isolated.

CI-9 : Colourless crystalline compound (60mg), soluble in pyridine, m.p. 306-8°C. Rf=0.70 (silica gel, benzene-acetone, 9:1). UV inactive, developed on charring with perchloric acid.

IR ν_{max}^{KBr} cm^{-1} : 3450, 3400, 2950, 2850, 1690, 1650, 1450, 1390, 1360, 1290, 1250, 1210, 1170, 1140, 1110, 1020, 1000, 980, 930, 910, 890, 850.

Me-ester of CI-9: **CI-9** was refluxed with $(CH_3)_2$ SO_4 and K_2CO_3 for 24h, crystalized in chloroform-ethanol to give colourless crystalline compound, soluble

in chloroform, m.p. 215-17°C. Rf =0.92 (silica gel, petrol-benzene, 2:8). Developed by absorption of iodine and charring with perchloric acid.

IR and ^{1}H NMR data of Cl-9 methyl ester

IR ν_{max}^{KBr} cm^{-1} : 2900, 1710, 1460, 1440, 1380, 1350, 1330, 1200, 1150, 1100, 1020.

^{1}H NMR (100 MH_z) : δ0.72 (s, 3H, Me), 0.88 (s, 3H, Me), 0.92 (s, 3H, Me), 0.96 (s, 3H, Me), 1.00 (s, 3H, Me), 1.04 (s, 3H, Me), 1.24-2.56 (m, methylene protons), 3.64 (s, 3H, - CO_2 Me).

Form IR and ^{1}H NMR spectral data of **Cl-9** and its methyl ester the compound was found to be **canophyllic acid**.

REFERENCES

1. Govindachari, T.R.; Viswanathan, N.; Pai, B.R.; Rao, V.R. and Srinivasan, M. *Tetrahedron* ***1967***, 23(4), 1901.

2. Kumar, V,; Ramachandran, S. and Sultanbawa, M.U.S. *Phytochemistry* ***1976***, 15 (12), 2016.

3. Goh, S.H.; Jantan, I. and Waterman, P. G. *J. Nat. Prod.* ***1992***, 55(10), 1415.

4. Sampath Kumar, P.S.; Murti, V. V. S.; Seshadri, T.R. and Trivenkate, R. *Indian J. Chem.* ***1970***, 8(1), 105.

5. Iinuma, M.; Tosa, H.; Tanaka, T. and Yonemori, S. *Phytochemistry* ***1994***, 35, 527.

6. Al jeboury, F.S. and Locksley, H.D. *Phytochemistry* ***1971***, 10(3), 603.

7. Govindachari, T.R.; Pai, B.R.; Muthukumaraswamy, N.; Rao, U.R. and Rao. N.N. *Indian J. Chem.* ***1968***, 6(2), 57.

8. Iinuma, M.; Tosa, H.; Tanaka, T. and Yonemori, S. *Phytochemistry* ***1995***, 38(3), 725.

9. Jackson, B.; Locksley, H.D. and Scheinmann, F. *Phytochemistry* ***1969***, 8(5), 927.

10. Sundaran, B.M.; Gopalkrishan, C. and Subramanian, S. *Arogya* Manipal, India ***1986***, 121 (1), 48.

11. Iinuma, M,; Tosa, H.; Tanaka, T. and Yonemori, S. *Heterocyles* ***1994***, 37, 833.

12. Games, D.E. *Tetrahedron Lett.* ***1972***, 31, 3187.

13. Adinarayana, D. and Seshadri. T.R. *Bull. Nat. Inst. Sci.* India ***1965***, 37, 91.

14. Polonsky, J. *Bull. Soc. Chim.* France ***1957***, 1079.

15. Mitra, C. *J. Sci. Ind. Res.* ***1957***, 16B, 120.

16. Patil, A.D.; Freyer, A.J.; Eggleston, D.S.; Haltiwanger, R.C.; Bean, M.F.; Taylor, P.B.; Caranfa, M.J.; Breen, A.L.; Bartus, H.R. et al. *J. Med. Chem.* ***1993***, 36(26), 4131.

17. Polonsky, J. *Cienc. Cult.* Sao Paulo ***1968***, 20,19.

18. Vernenghi, A.; Ramiandrasoa, F.; Chuilon, S. and Ravise, A. *Fruit* ***1987***, 42, 103.

19. Bhushan, B.; Rangaswamy, S. and Seshadri, T.R. *Indian J. Chem,* ***1975***. 13 (7), 746.

20. Kawazu, K.; Ohigashi, H. and Mitsui, T. *Tetrahedron Lett.* ***1968***, 19, 2383.

21. Kawazu, K.; Ohigashi, H; Takahashi, N. and Mitsui, T. *Bull. Inst. Chem. Res.* Kyoto Uni. ***1972***, 50(3), 160.

22. Subramanian, S.S. and Nair, A.G.R. *Phytochemistry* ***1971***, 10(7), 1679.

23. Subramanian, S,S, and Nair. A.G.R. *Bull. Nat. Inst. Sci.* India ***1965***, 31, 39.

24. Goh, S.H.; Jantan, I.; Wei, C. and Mak, T.C.W. *Nat. Prod. Lett.* ***1993***, 2(3), 191.

25. Gautier, J.; Kunesch. G. and Polonsky, J. *Tetrahedron Lett.* ***1972***, 27,

2715.

26. Stout, G.H.; Krahn, M.M. and Breck. G.D. *Tetrahedron Lett.* ***1968***, 29, 3285.

27. Hoefle, G.; Steglich, W. and Vorbriieggen, H. *Ang. Chem.* ***1978***, 90, 602.

28. Arnone, A.; Cardillo, G.; Merlini, I. and Mondelli, R. *Tetrahedron Lett.* ***1967***, 4201.

29. Guerriro, E.; Kunesch, G. and Polonsky, J. *Phytochemistry* ***1971***, 10, 2139.

30. Nomura, T. and Fukai, T. *Heterocycles* ***1979***, 12, 1289.

31. Agrawal, P.K. and Rastogy, R.P. *Heterocycles* ***1981***, 16, 2181.

32. Babu, V.; Arya, R.; Ilyas, M. and Nasim, K.T. *Phytochemistry* ***1994***, 35, 507.

33. Gaffield, W. *Tetrahedron* ***1970***, 26, 4093.

34. Scopes, P.M. In *Fortschritte der Chemie Organischer Naturstoffe*, Vol. 32 (eds Herz, W.; Grisebach, H. and Kirby, G.W.; Springer-Verlag, New York, ***1975***, p.167.

CHAPTER - II

THE CONSTITUENTS OF THE LEAVES OF *MURRAYA KOENIGII (RUTACEAE).*

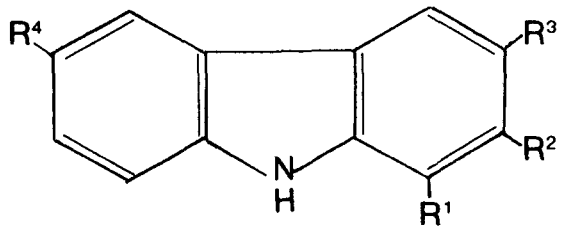
INTRODUCTION

1. CARBAZOLE ALKALOIDS

(a) Carbazole alkaloids containing secondary nitrogen :

Tricyclic : Tricyclic carbazole alkaloids isolated from stem bark of *M. koenigii* were mukoeic acid (**Ia**)[1,2], mukonal (**Ib**)[3], 1-hydroxy-3-methyl carbazole (**Ic**)[4] and me-2-methoxy carbazole-3-carboxylate(**Id**)[4]. Koenoline (**Ie**) isolated from root bark of *M. koenigii* exhibited cytotoxic activity against the KB cell-culture test system[5]. 2-Methoxy-3-methyl carbazole (**If**)[6] from stem bark and murrayanine (**Ig**)[7,8] from leaves and stem bark of *M. koenigii* were also isolated. Other tricyclic carbazole alkaloids isolated from *M. koenigii* were mukonidine (**Ih**)[9], mukoline (**Ii**)[10], mukolidine (**Ij**)[10], mukonine (**Ik**)[11], 2-hydroxy-3-methyl carbazole (**Il**)[12], 3-methyl carbazole (**Im**)[13], glycozoline[13], mukoenine-A, -B and -D[14]. The tricyclic carbazole alkaloids having branched side chain, isolated from the stem bark and seeds of *M. koenigii*, were grinimbilol (**II**)[15], mahanimbinol = mahanimbilol (**IIIa**)[8,15] and murrayanol (**IIIb**)[16].

Tetracyclic : Murrayacine (**IVa**)[17,18] and koenidine = koenigicine = koenimbidine (**IVb**)[17,19-21] from leaves and roots, grinimbine (**IVc**)[17,22] from stem bark, leaves and roots, koenimbin = koenimbine (**IVd**)[19,23] from fruits, mukonicine (**IVe**)[24] from leaves along with koenine (**IVf**)[19,21] and koenigine (**IVg**)[19,21] were isolated from *M. koenigii* having no side chain. However tetracyclic carbazole alkaloids containing branched side chain included mahanimbine (**Va**)[17,19,23,25-28] from fruits and leaves, mahanimbicine = isomahanimbin (**Vb**)[17,19,29] from roots and leaves, isomahanine (**Vc**)[16] from seeds, mahanine (**Vd**)[19,21,30] from leaves and



I

a $R^2 = R^4 = H,\ R^1 = OCH_3,\ R^3 = CO_2H$

b $R^1 = R^4 = H,\ R^2 = OH,\ R^3 = CHO$

c $R^2 = R^4 = H,\ R^1 = OH,\ R^3 = CH_3$

d $R^1 = R^4 = H,\ R^2 = OCH_3,\ R^3 = CO_2CH_3$

e $R^2 = R^4 = H,\ R^1 = OCH_3,\ R^3 = CH_2OH$

f $R^1 = R^4 = H,\ R^2 = OCH_3,\ R^3 = CH_3$

g $R^2 = R^4 = H,\ R^1 = OCH_3,\ R^3 = CHO$

h $R^1 = R^4 = H,\ R^2 = OH,\ R^3 = CO_2CH_3$

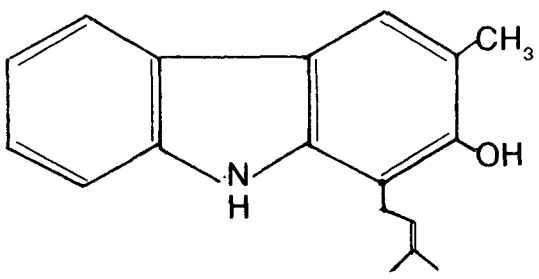
i $R^2 = R^3 = H,\ R^1 = OCH_3,\ R^4 = CH_2OH$

j $R^2 = R^3 = H,\ R^1 = OCH_3,\ R^4 = CHO$

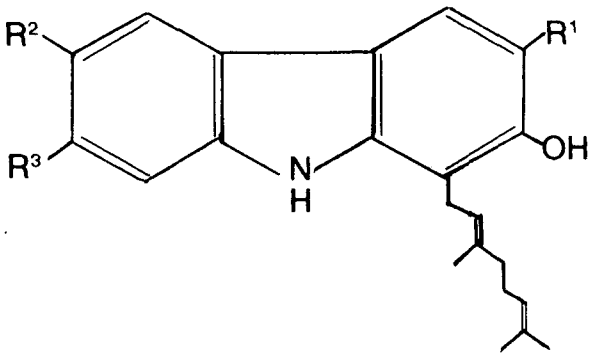
k $R^2 = R^4 = H,\ R^1 = OCH_3,\ R^3 = CO_2CH_3$

l $R^1 = R^4 = H,\ R^2 = OH,\ R^3 = CH_3$

m $R^1 = R^2 = R^4 = H,\ R^3 = CH_3$



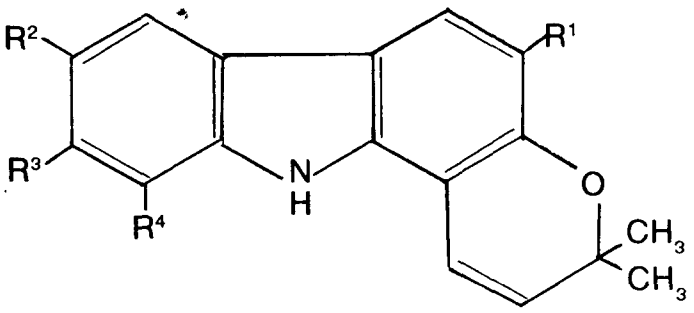
II



III

a $R^2 = R^3 = H,\ R^1 = CH_3$

b $R^1 = H,\ R^2 = CH_3,\ R^3 = OH$



IV

a $R^1 = R^3 = R^4 = H,\ R^2 = CHO$

b $R^4 = H,\ R^1 = CH_3,\ R^2 = R^3 = OCH_3$

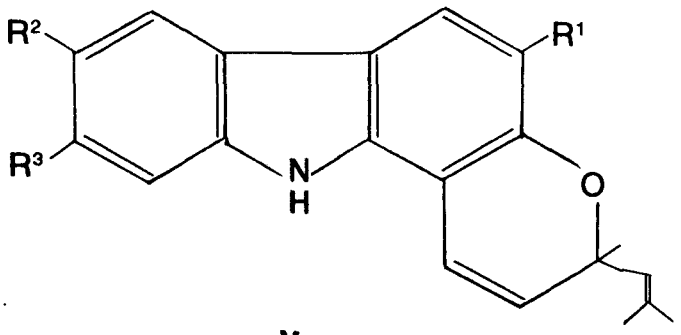
c $R^2 = R^3 = R^4 = H,\ R^1 = CH_3$

d $R^3 = R^4 = H,\ R^1 = CH_3,\ R^2 = OCH_3$

e $R^3 = H,\ R^1 = CH_3,\ R^2 = R^4 = OCH_3$

f $R^3 = R^4 = H,\ R^1 = CH_3,\ R^2 = OH$

g $R^4 = H,\ R^1 = CH_3,\ R^2 = OCH_3,\ R^3 = OH$



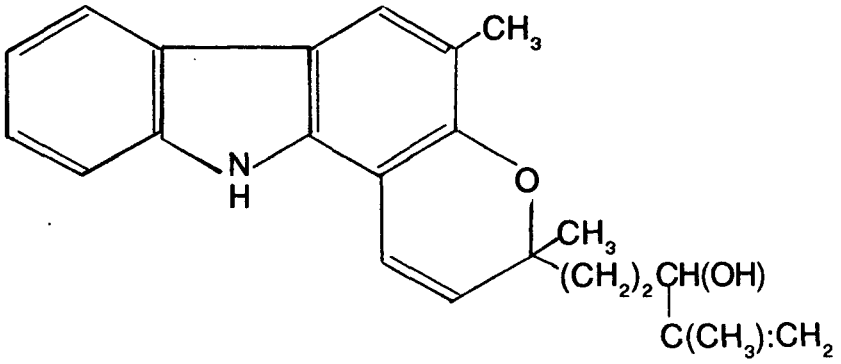
V

a $R^1 = R^2 = R^3 = H$

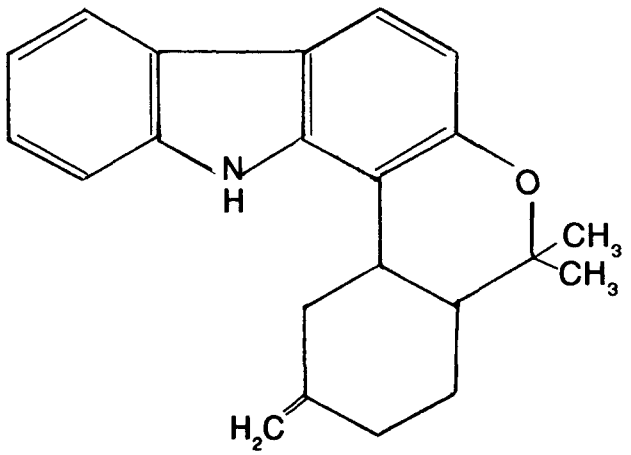
b $R^1 = R^3 = H$, $R^2 = CH_3$

c $R^1 = H$, $R^2 = CH_3$, $R^3 = OH$

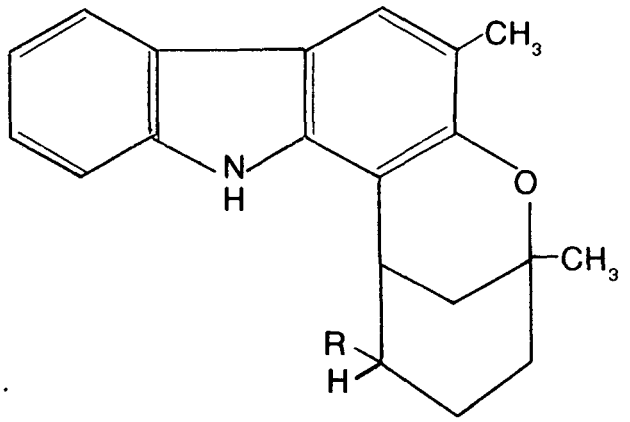
d $R^2 = H$, $R^1 = CH_3$, $R^3 = OH$



VI



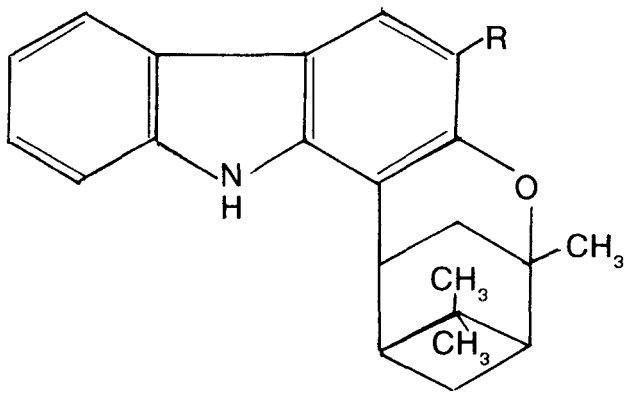
VII



VIII

a R = $C(CH_3)_2OH$

b R = $C(CH_3)CH_2$



IX

a R = H

b R = CH_3

mahanimboline (**VI**)[31] were also isolated from *M. koenigii.*

Pentacyclic : The only pentacyclic carbazole alkaloid without any side chain isolated from stem bark of *M. koenigii* was murrayazolidine (**VII**)[32] while murrazolinine (**VIIIa**)[33] and cyclomahanimbin = currayanine (**VIIIb**)[27,34] isolated from leaves, possess branched side chain.

Hexacyclic : Bicyclomahanimbicine (**IXa**)[29] from leaves and bicyclomahanimbin (**IXb**)[8,27] from both leaves and stem bark of *M. koenigii* were isolated.

Carbazole alkaloids murrayanine, grinimbine and mahanimbine containing secondary nitrogen isolated from stem bark of *M. koenigii* also showed antifungal activity against some human pathogenic fungii[35].

(b) Carbazole alkaloids containing tertiary nitrogen :

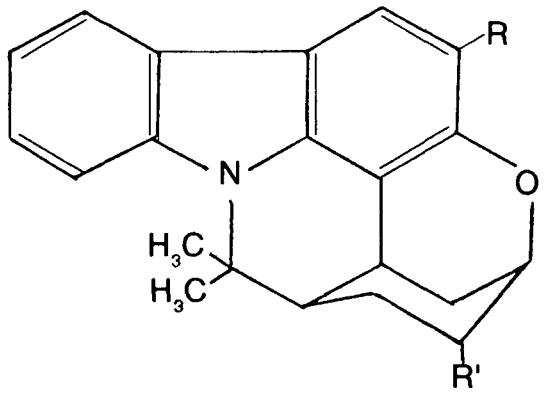
Isomurrayazoline (**Xa**)[36], currayangine = mahanimbidine = murrazoline (**Xb**)[8,25,27,34] from leaves and stem bark while murrazolinol (**Xc**)[37] from stem bark of *M. koenigii* were isolated. The crystallographic parameters for murrayazoline[38] are a 11.19, b 11.05, c 15.65 é, β 115.27°, d. (exptl.) = 1.24, z = 4, d. (calcd.) = 1.258, space group P21/c, R = 0.074.

(c) Binary carbazole alkaloids and quinones :

Binary carbazole alkaloids and quinones isolated from *M. koenigii* included murrastifoline - F, bis-2-hydroxy-3-methyl carbazole, bismahanine, biskoeniquinone-A and bis murrayaquinone-A[14].

2. ESSENTIALOILS

α- pinene[39,40], l-caryophylene[39], sabinene[39], β-caryophyllene[41], β-elemene[41], β-phellandrene[41], terpenen-4-ol[40] from leaves while 8-

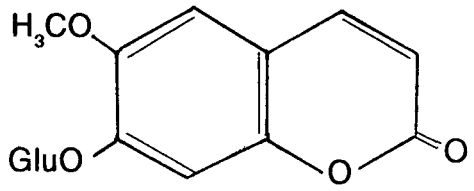


X

a R = H, R' = H

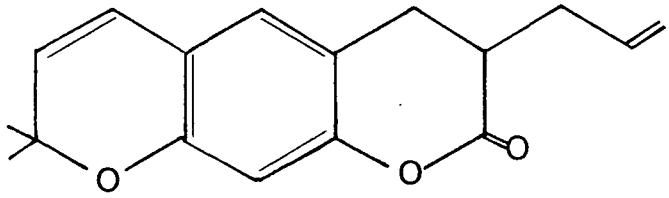
b R = CH_3, R' = H

c R = CH_3, R' = OH

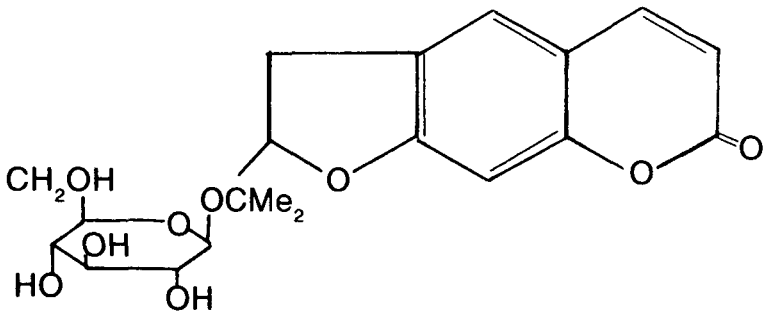


XI

Glu = glucopyranosyl



XII



XIII

geranyloxypsoralene[42], imperatorin[42], heraclenin[42], isosaxalin[42] and heraclenol[42] were isolated from the seeds of *M. koenigii*.

3. MISCELLANEOUS

Glycoside scopoline (**XI**)[7], glucose[7] from leaves and 3-(1,2-dimethyl allyl) xanthyletin (**XII**)[43] from stem bark of *M. koenigii* were isolated. Marmesin-1"-O-β-D-galactopyranoside (**XIII**) and umbelliferone isolated from stem bark of *M. koenigii* showed antiinflammatory and antimicrobial activities[44].

RESULTS AND DISCUSSION

The dried leaves of *Murraya koenigii* were extracted with methanol and methanol extract was concentrated and treated with petrol, benzene, ethyl acetate and acetone. The petrol fraction on column chromatography using silica gel as adsorbent and eluting with petrol-benzene (1:1), yielded a crystalline product **MK-1**.

MK-1, crystallized from benzene, m.p. 205°C, gave brown fluorescence in UV light after developing on thin layer chromatography. In the infra red spectrum bands appeared at 3400 (N-H stretching), 1640, 1610 and 1580 cm^{-1} (unsaturation and aromatic system). It analysed for $C_{19}H_{19}NO_2$ (M^+ 293). In the mass spectrum the molecular ion appeared at m/z 293 and base peak appeared at m/z 278 ($M-CH_3$). Other prominent peaks appeared at m/z 235 (M-58) and m/z 139. The odd mass number for molecular ion is indicative of the presence of nitrogen.

In the ^1H NMR spectrum (600 MHz, $CDCl_3$) given in Table 1 and Fig.1, a singlet integrating for six protons at δ 1.485 can be assigned to gem dimethyl protons. The singlet at δ 2.340 is attributable to benzylic methyl group while a singlet at δ 3.903 can be assigned to methoxy protons. A pair of doublet appearing at δ 5.640 (J=10 Hz) and 6.536 (J=10 Hz) were assigned to 3'-H and 4'-H, respectively, of chromene ring. The spectrum shows signals for aromatic protons at δ 7.627 (s, 4-H), 6.940 (dd, J=8.4, 2.2 Hz, 7-H), 7.214 (d, J=8.4 Hz, 8-H) and 7.421 (d, J=2.2 Hz, 5-H). The broad singlet at δ 7.686 was assigned to N-H proton. The assignments were supported by DEPT experiments (Fig. 2) and HHCOSY correlations (Fig. 3).

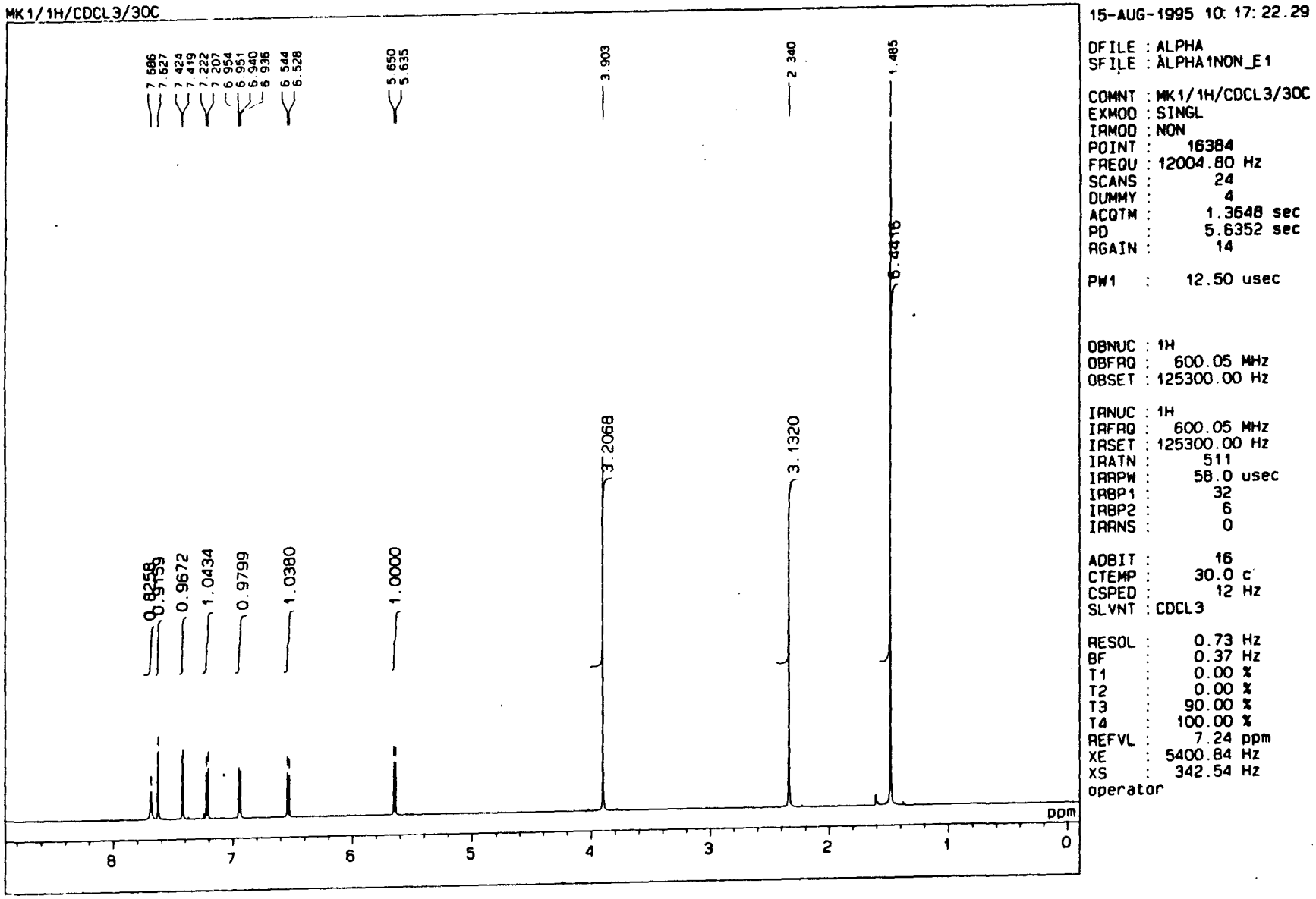

MK1/1H/CDCL3/30C
7.686
7.627
7.424
7.419
7.222
7.207
6.954
6.951
6.940
6.936
6.544
6.528
5.650
5.635
3.903
2.340
1.485
6.4416
3.2068
3.1320
0.8258
0.9159
0.9672
1.0434
0.9799
1.0380
1.0000
ppm
8
7
6
5
4
3
2
1
0
15-AUG-1995 10: 17: 22.29
DFILE : ALPHA
SFILE : ALPHA1NON_E1
COMNT : MK1/1H/CDCL3/30C
EXMOD : SINGL
IRMOD : NON
POINT : 16384
FREQU : 12004.80 Hz
SCANS : 24
DUMMY : 4
ACQTM : 1.3648 sec
PD : 5.6352 sec
RGAIN : 14
PW1 : 12.50 usec
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125300.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 12 Hz
SLVNT : CDCL3
RESOL : 0.73 Hz
BF : 0.37 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 7.24 ppm
XE : 5400.84 Hz
XS : 342.54 Hz
operator

Fig.1

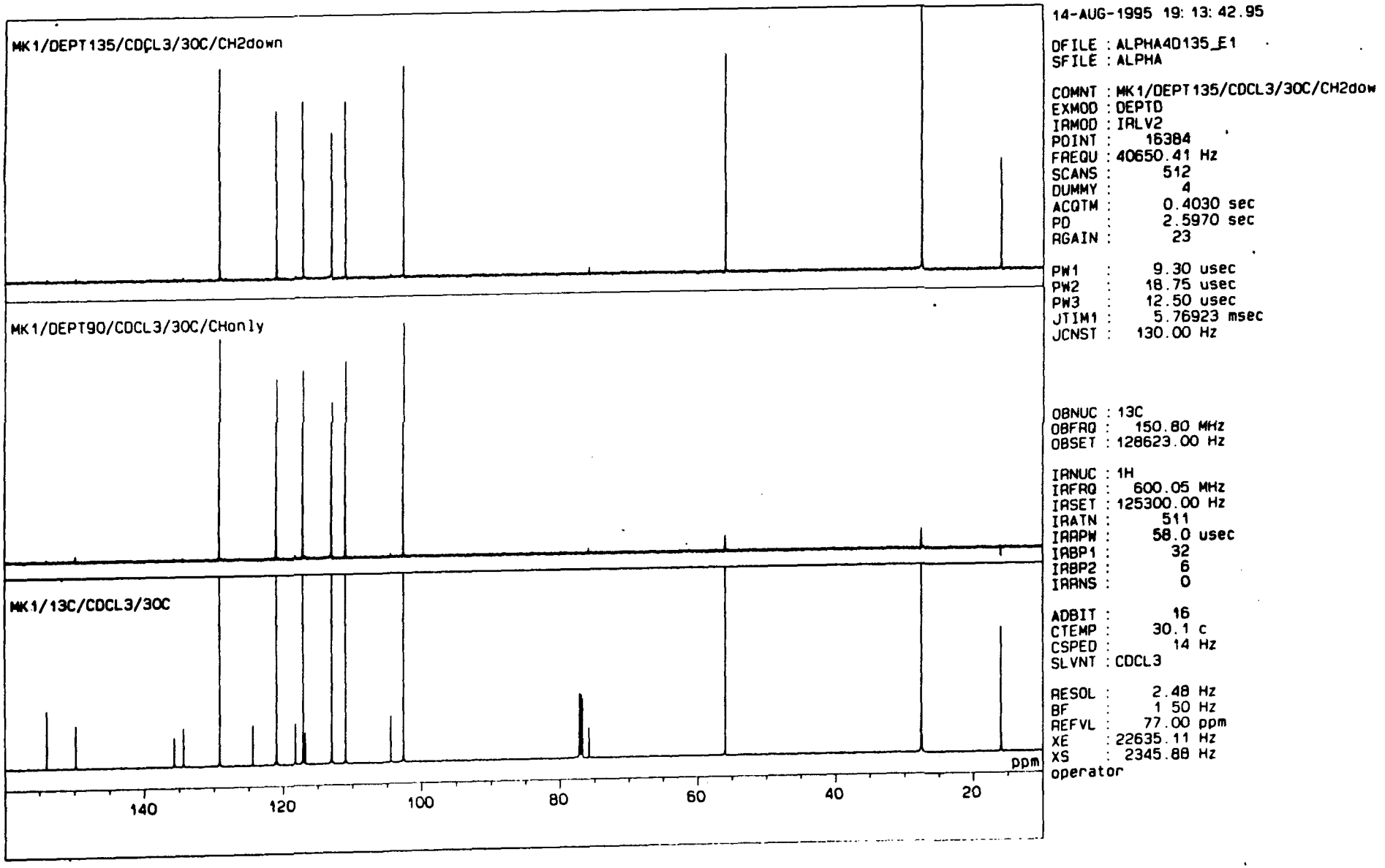

MK1/DEPT135/CDCL3/30C/CH2down
MK1/DEPT90/CDCL3/30C/CHonly
MK1/13C/CDCL3/30C
ppm
140
120
100
80
60
40
20
14-AUG-1995 19: 13: 42.95
DFILE : ALPHA4D135_E1
SFILE : ALPHA
COMNT : MK1/DEPT135/CDCL3/30C/CH2dow
EXMOD : DEPTD
IRMOD : IRLV2
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 512
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23
PW1 : 9.30 usec
PW2 : 18.75 usec
PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRANS : 0
ADBIT : 16
CTEMP : 30.1 c
CSPED : 14 Hz
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 1 50 Hz
REFVL : 77.00 ppm
XE : 22635.11 Hz
XS : 2345.88 Hz
operator

Fig.2

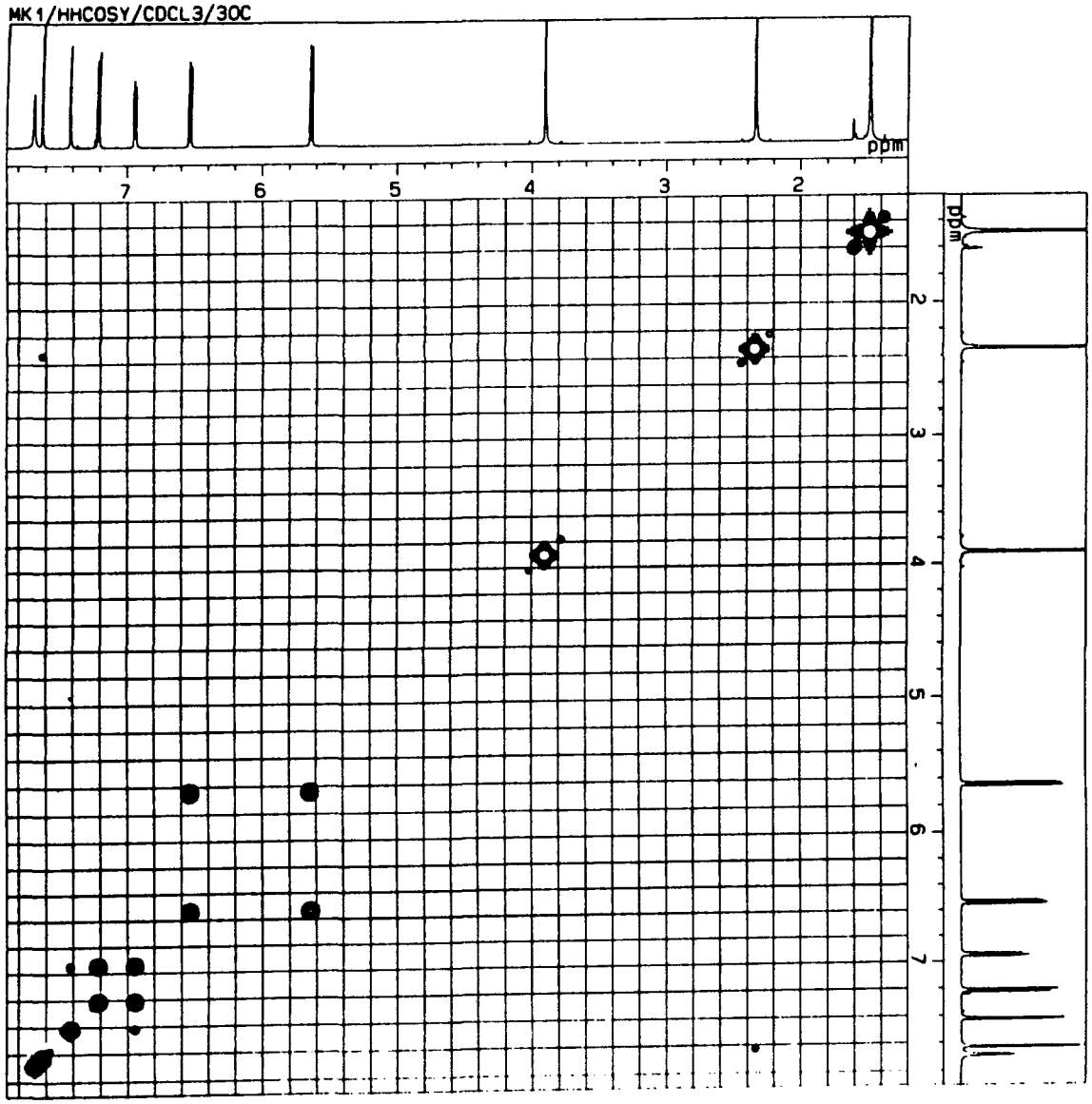


Fig.3

15-AUG-1995 10:25:32.41

DFILE : ALPHA
SFILE : ALPHA2COSY_E1
HR2FILE: ALPHA1NON_E1
HR1FILE: ALPHA1NON_E1

COMNT : MK1/HHCOSY/CDCL3/30C
EXMOD : COSY
IRMOD : NON
POINT : 512
FREQU : 4545.45 Hz
SCANS : 8
DUMMY : 4
ACQTM : 0.0563 sec
PD : 0.9437 sec
RGAIN : 11

CLFRQ : 4545.45 Hz
CLPNT : 512
TOSCN : 256
CINWT : 10.00 usec
CINTV : 220.00 usec

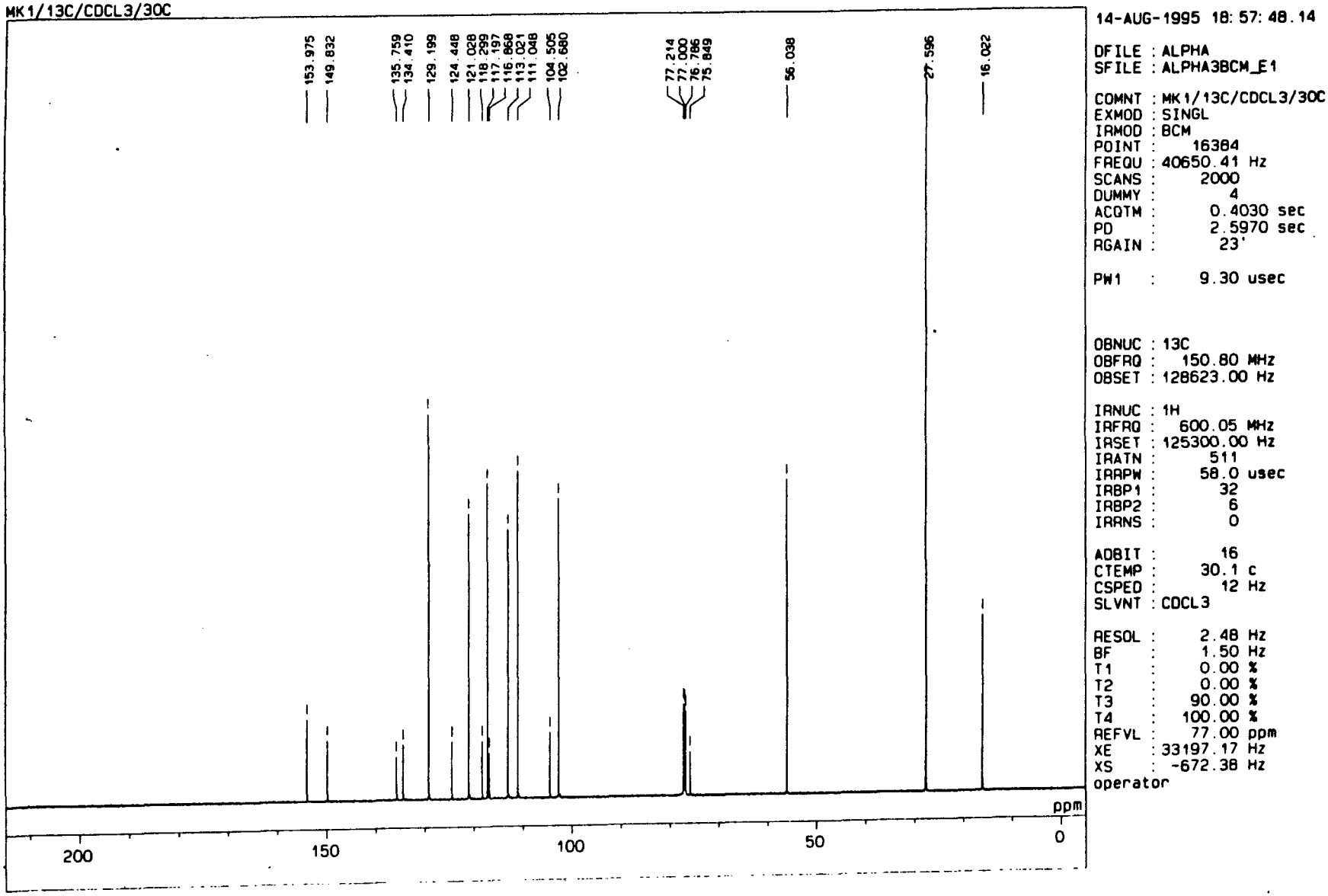
PW1 : 12.50 usec
PW2 : 20.00 usec
PI1 : 120.0000 msec
PI2 : 1.0000 msec

OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125036.96 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRNS : 0

ADBIT : 16
CTEMP : 30.0 c
CSPED : 12 Hz
SLVNT : CDCL3

RESOL : 8.88 Hz
CLRSO : 8.88 Hz
TLINE : 16
THTOP : 30.0000
THBTM : 0.6000
operator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149.832
135.759
134.410
129.199
124.448
121.028
118.299
117.197
116.868
113.021
111.048
104.505
102.680
77.214
77.000
76.786
75.849
56.038
27.596
16.022
ppm
200
150
100
50
0
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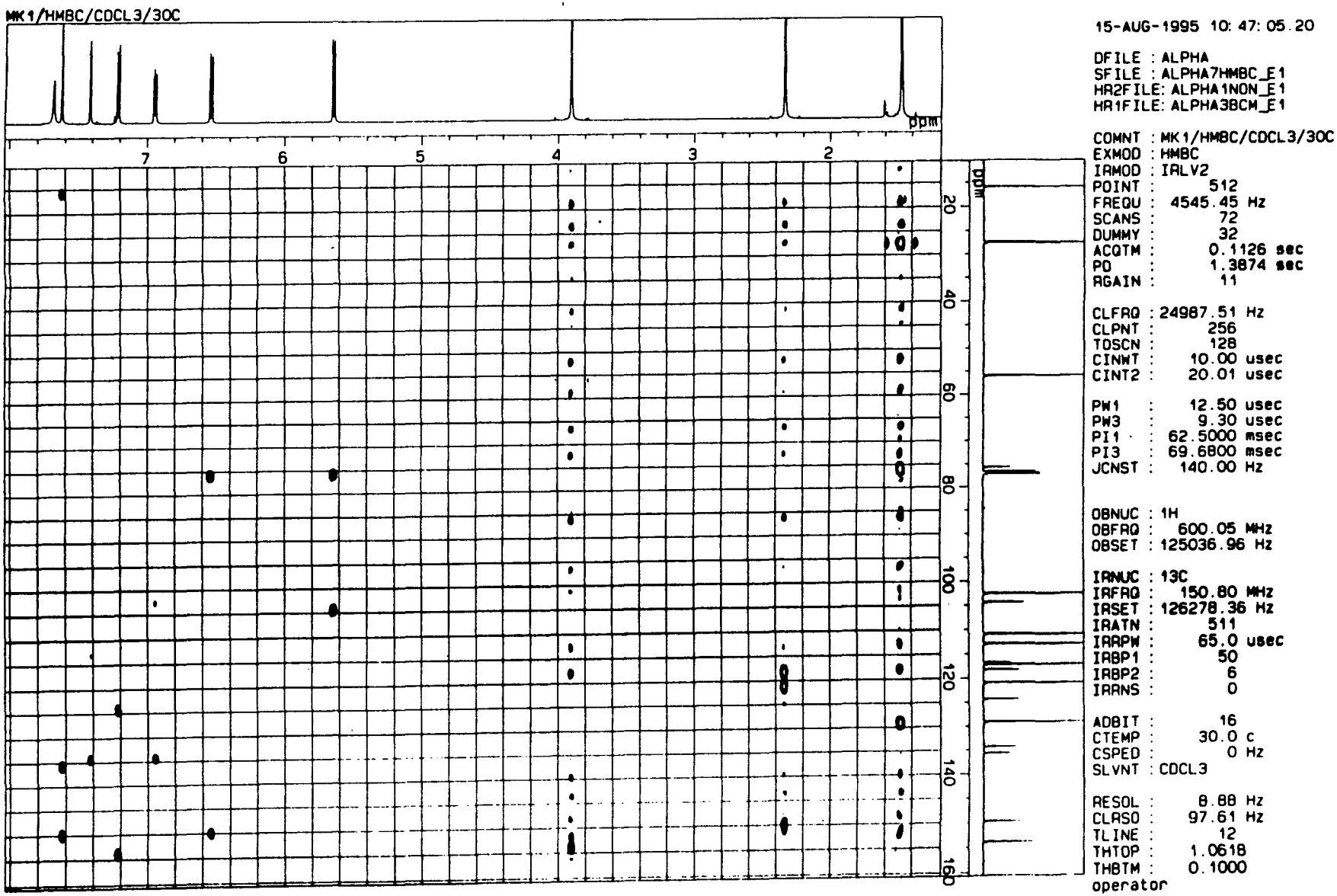
SFILE : ALPHA3BCM_E1
COMNT : MK1/13C/CDCL3/30C
EXMOD : SINGL
IRMOD : BCM
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 2000
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23'
PW1 : 9.30 usec
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.1 c
CSPED : 12 Hz
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 1.50 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 77.00 ppm
XE : 33197.17 Hz
XS : -672.38 Hz
operator

Fig.4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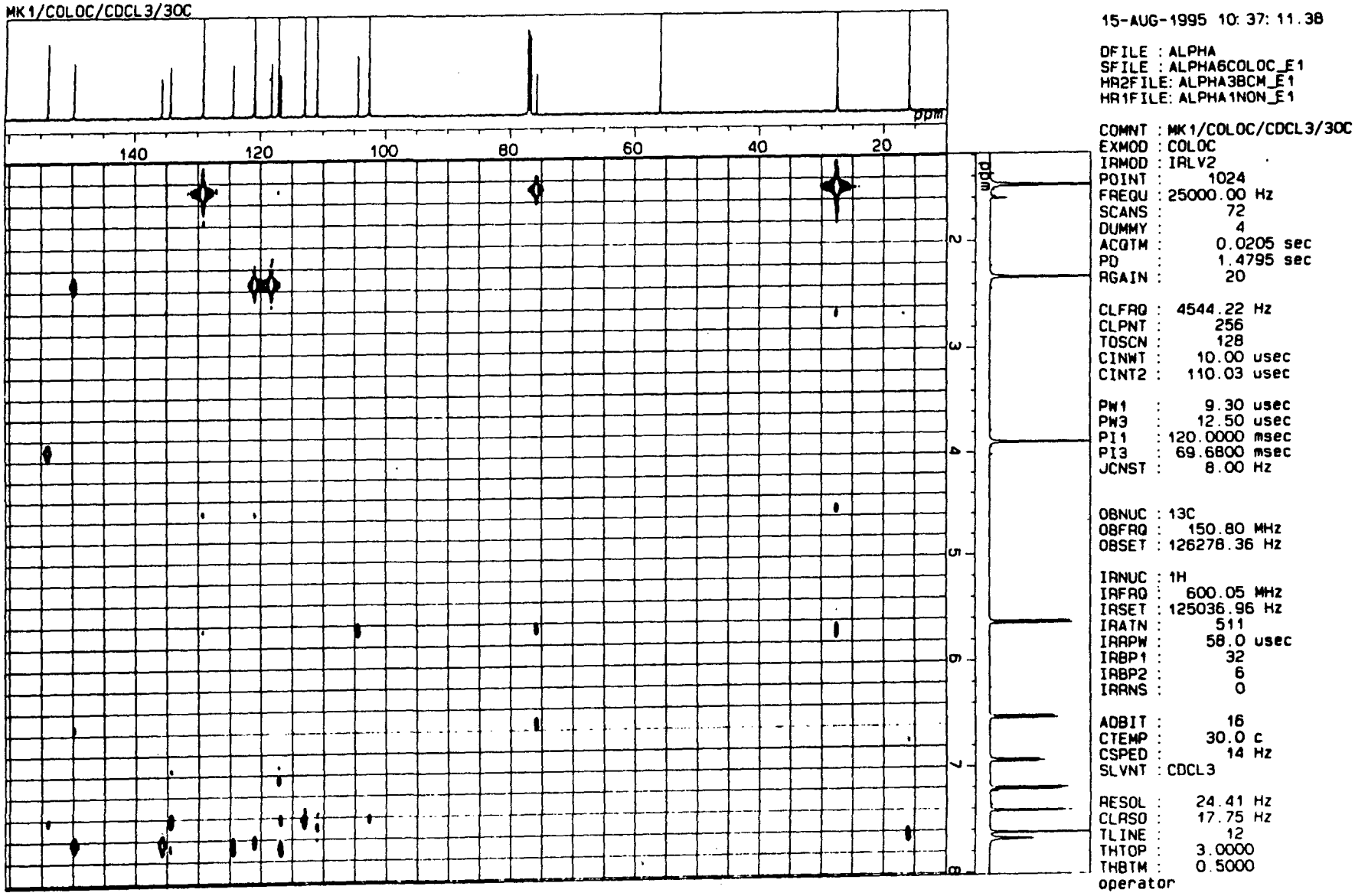
6
5
4
3
2
20
40
60
80
100
120
140
160
ppm
15-AUG-1995 10:47:05.20
DFILE : ALPHA
SFILE : ALPHA7HMBC_E1
HR2FILE: ALPHA1NON_E1
HR1FILE: ALPHA3BCM_E1
COMNT : MK1/HMBC/CDCL3/30C
EXMOD : HMBC
IRMOD : IRLV2
POINT : 512
FREQU : 4545.45 Hz
SCANS : 72
DUMMY : 32
ACQTM : 0.1126 sec
PD : 1.3874 sec
RGAIN : 11
CLFRQ : 24987.51 Hz
CLPNT : 256
TDSCN : 128
CINWT : 10.00 usec
CINT2 : 20.01 usec
PW1 : 12.50 usec
PW3 : 9.30 usec
PI1 : 62.5000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125036.96 Hz
IRNUC : 13C
IRFRQ : 150.80 MHz
IRSET : 126278.36 Hz
IRATN : 511
IRRPW : 65.0 usec
IRBP1 : 50
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 0 Hz
SLVNT : CDCL3
RESOL : 8.88 Hz
CLRSO : 97.61 Hz
TLINE : 12
THTOP : 1.0618
THBTM : 0.1000
operator

Fig.5


MK1/COLOC/CDCL3/30C
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120
100
80
60
40
20
2
3
4
5
6
7
8
15-AUG-1995 10:37:11.38
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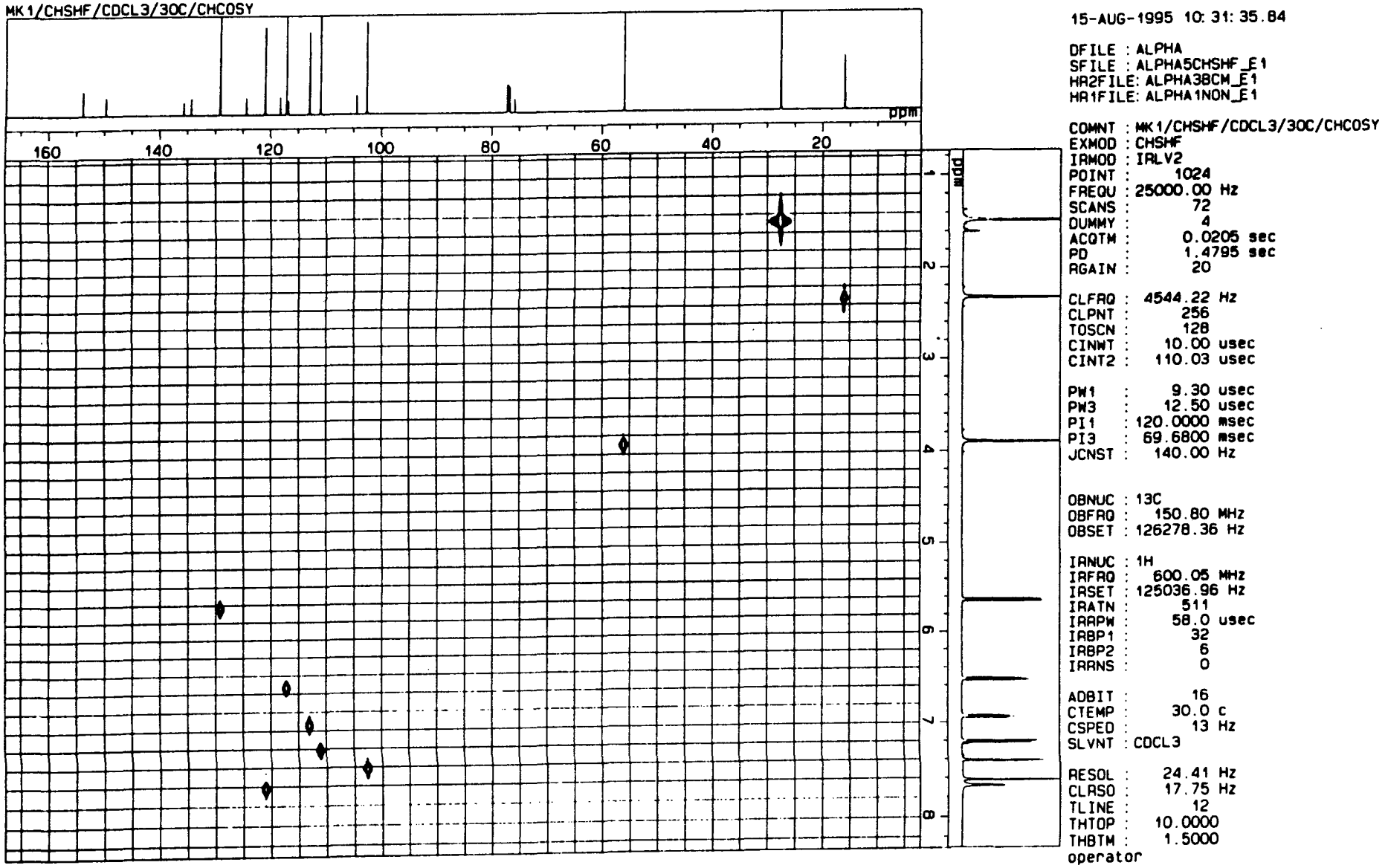
SFILE : ALPHA6COLOC_E1
HR2FILE: ALPHA3BCM_E1
HR1FILE: ALPHA1NON_E1
COMNT : MK1/COLOC/CDCL3/30C
EXMOD : COLOC
IRMOD : IRLV2
POINT : 1024
FREQU : 25000.00 Hz
SCANS : 72
DUMMY : 4
ACQTM : 0.0205 sec
PD : 1.4795 sec
RGAIN : 20
CLFRQ : 4544.22 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 110.03 usec
PW1 : 9.30 usec
PW3 : 12.50 usec
PI1 : 120.0000 msec
PI3 : 69.6800 msec
JCNST : 8.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 126278.36 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125036.96 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 14 Hz
SLVNT : CDCL3
RESOL : 24.41 Hz
CLRSO : 17.75 Hz
TLINE : 12
THTOP : 3.0000
THBTM : 0.5000
operator

Fig.6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140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
ppm
15-AUG-1995 10:31:35.84
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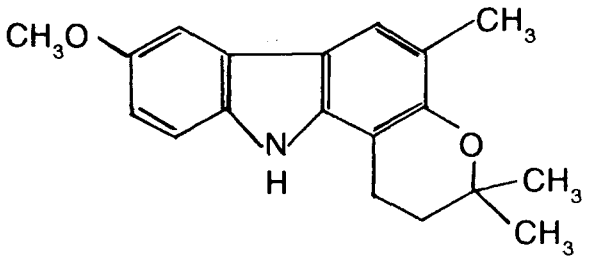
HR2FILE: ALPHA3BCM_E1
HR1FILE: ALPHA1NON_E1
COMNT : MK1/CHSHF/CDCL3/30C/CHCOSY
EXMOD : CHSHF
IRMOD : IRLV2
POINT : 1024
FREQU : 25000.00 Hz
SCANS : 72
DUMMY : 4
ACQTM : 0.0205 sec
PD : 1.4795 sec
RGAIN : 20
CLFRQ : 4544.22 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 110.03 usec
PW1 : 9.30 usec
PW3 : 12.50 usec
PI1 : 120.0000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 126278.36 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125036.96 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRANS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 24.41 Hz
CLRSO : 17.75 Hz
TLINE : 12
THTOP : 10.0000
THBTM : 1.5000
operator

Fig.7

The ^{13}C NMR spectrum (150.8 MHz, $CDCl_3$) given in Table I and Fig. 4,shows signals for all the 19 carbons. The signal at δ16.022 was assigned to methyl carbon C-3 of carbazole nucleus which was supported by DEPT experiments. The geminal methyl group carbons at C-2' absorb at δ 27.596 while methoxy carbon attached to C-6 gives signal at δ56.038 and C-2' absorb at δ 75.849 which was confirmed by DEPT experiments. The ^{13}C NMR signals for sp^2 carbons in the carbazole skeleton are as follows δ102.680 (C-5), 104.505 (C-1), 111.048 (C-8), 113.021 (C-7), 116.868 (C-4b), 117.197 (C-4'), 118.299 (C-3), 121.028 (C-4), 124.448 (C-4a), 129.199 (C-3'), 134.410 (C-8a), 135.759 (C-9a), 149.832 (C-2), 153.975 (C-6). The assignments were further confirmed by DEPT experiments, HMBC (Fig. 5), COLOC (Fig.6), and CHCOSY (Fig.7) correlations. On the basis of above data **MK-1** was confirmed as **koenimbine** (**IVd**) m.p. 205° C (lit. 194-95° C)[19,23] which was characterized earlier on the basis of ^1H NMR, IR, UV and MS only. The ^{13}C NMR and 2D NMR is discussed for the first time.



IVd

Further elution of the column with petrol - benzene and crystallization from benzene gave colourless crystals of **MK-2.**

MK-2, m.p. 139-40°C was UV inactive and it was detected by charring of TLC plate (SiO_2) with perchloric acid. In the IR spectrum a broad band at 3400

Table I. ^{1}H NMR and ^{13}C NMR data of MK-1 (IVd)

Assignment	Chemical Shift (δ,ppm)	
C/H	^{13}C NMR (150.8 MHz, $CDCl_3$)	^{1}H NMR (600MHz, $CDCl_3$)
1	104.505	-
2	149.832	-
3	118.299	-
4	121.028	7.627 (s)
4a	124.448	-
4b	116.868	-
5	102.680	7.421 (d, J=2.2 Hz)
6	153.975	-
7	113.021	6.940(dd,J=8.4,2.2 Hz)
8	111.048	7.214(d, J=8.4 Hz)
8a	134.410	-
9	-	7.686 (br,s)
9a	135.759	-
3-Me	16.022	2.340 (s)
6-OMe	56.038	3.903 (s)
1'	-	-
2'	75.849	-
3'	129.199	5.640 (d,J=10Hz)
4'	117.197	6.536 (d,J=10 Hz)
2'-Me_2	27.596	1.485 (s)

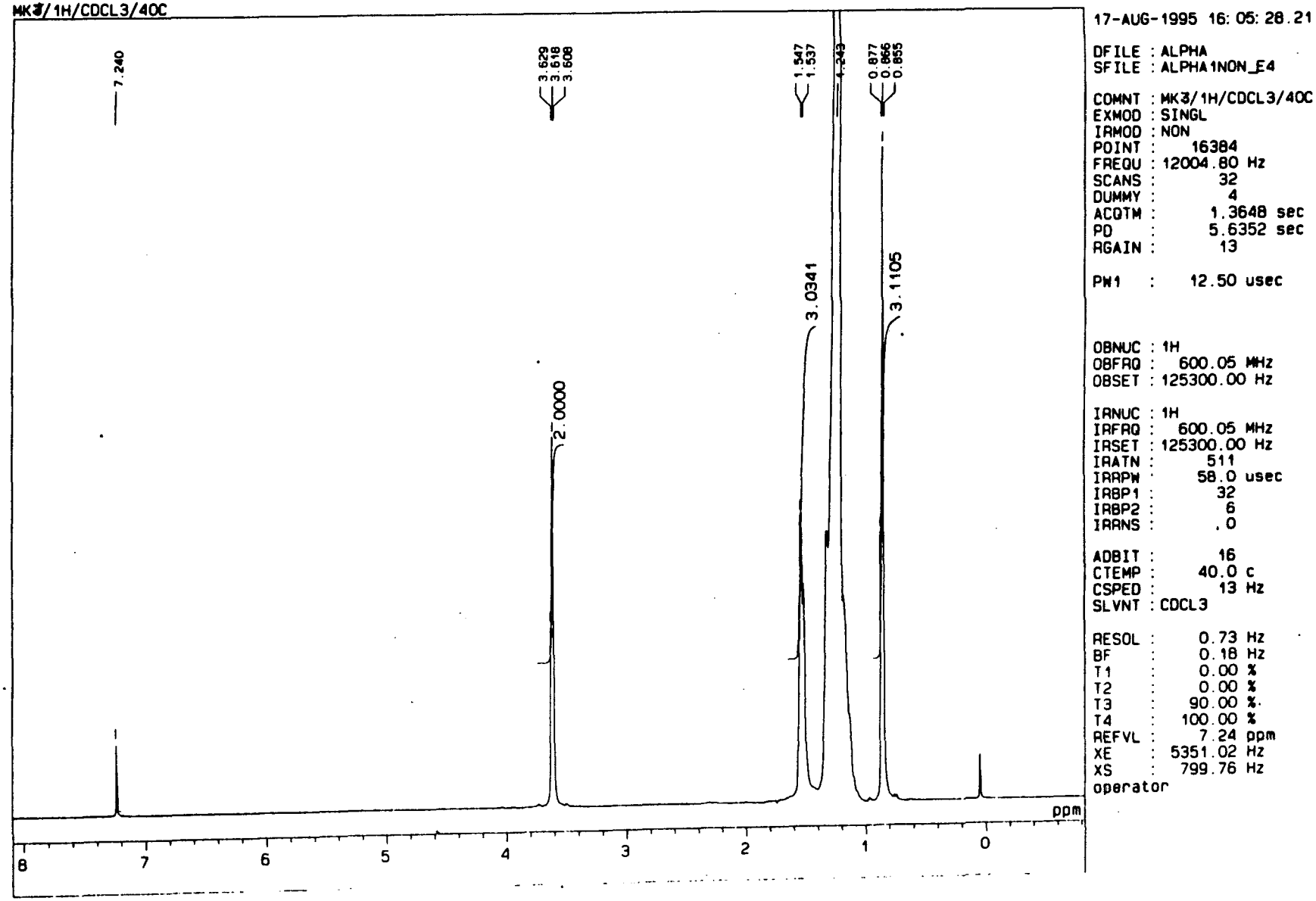
cm^{-1} indicated the presence of -OH group and the other bands were comparable with the IR spectrum of authentic sample of **β-sitosterol**[45]. The occurrence of **β-sitosterol** from *M. koenigii* has not been reported so far.

Elution of the column with benzene followed by preparative thin layer chromatography yielded **MK-3** and **MK-4.**

MK-3 was crystallized as colourless granular compound from alcohol - benzene (7:3) melting at 94.5°C. It was UV inactive and its homogeniety was checked on TLC plate (SiO_2) with iodine as well as perchloric acid. In the mass spectrum, molecular ion appeared at m/z 476 with successive loss of 14 showing peaks at m/z 462, 448, 434, 406 etc. and base peak appearing at 57, indicating the presence of **long chain hydrocarbon**.

The ^1H NMR spectrum (600 MHz, $CDCl_3$) is given in Fig. 8. It shows signals at δ 0.866 (t, J=6.6 Hz), 1.243 (s), 1.542 (d, J=5.88) and 3.618 integrating in the ratio 3 : 66 : 3 : 2 respectively. The consideration of ^1H NMR integration of 74 protons and the signal at δ 3.618 for two protons can lead to a molecular ion $C_{36}H_{74}O$ (M^+522), but the molecular ion peak at m/z 522 is not indicated in MS. In MS, a fairly unstable peak appeared at m/z 476 (3.5) as molecular ion peak.

The ^{13}C NMR spectrum (150.8 MHz, $CDCl_3$), given in Fig. 9, shows signals at δ 14.065 ($-CH_3$), 22.680 ($-CH_2-$), 25.771 ($-CH_2-$), 29.355 ($-CH_2-$), 29.454 ($-CH_2-$), 29.618 ($-CH_2-$), 29.700 $(-CH-)_n$, 31.936 ($-CH_2-$), 32.873 ($-CH_2-$) and 63.108 ($-CH_2O-$). The appearance of ^{13}C NMR signal at δ 63.108 and proton signal at δ 3.618 indicates the presence of CH_2 group attached to oxygen, probably an alcohol. The assignment of CH_2 and CH_3 carbons has been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7.240
3.629
3.618
3.608
1.547
1.537
1.243
0.877
0.866
0.855
2.0000
3.0341
3.1105
ppm
8
7
6
5
4
3
2
1
0
17-AUG-1995 16:05:28.21
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SFILE : ALPHA1NON_E4
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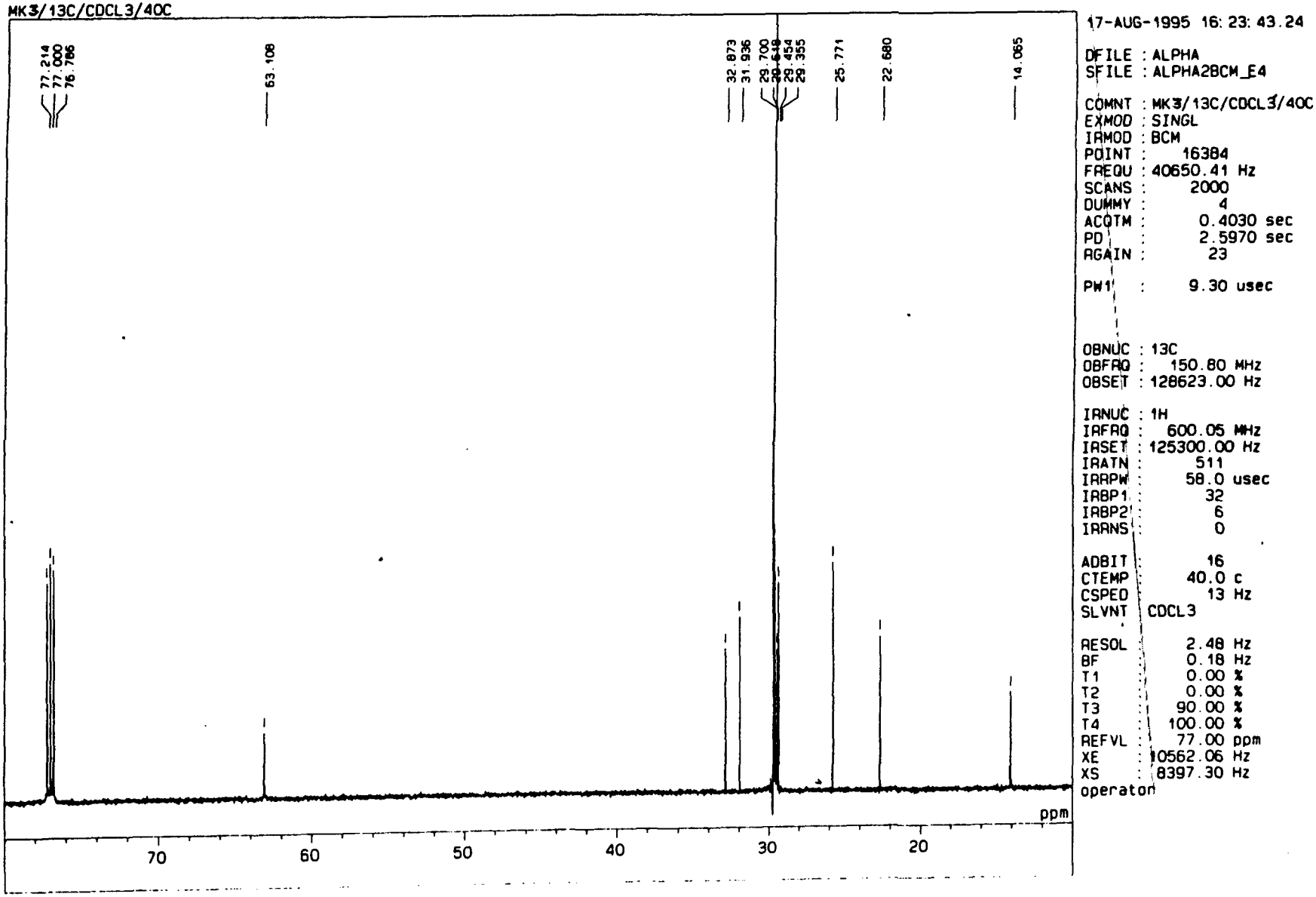
IRMOD : NON
POINT : 16384
FREQU : 12004.80 Hz
SCANS : 32
DUMMY : 4
ACQTM : 1.3648 sec
PD : 5.6352 sec
RGAIN : 13
PW1 : 12.50 usec
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125300.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : .0
ADBIT : 16
CTEMP : 40.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 0.73 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 7.24 ppm
XE : 5351.02 Hz
XS : 799.76 Hz
operator

Fig.8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77.000
76.786
63.108
32.873
31.936
29.700
29.648
29.454
29.355
25.771
22.680
14.065
ppm
70
60
50
40
30
20
17-AUG-1995 16:23:43.24
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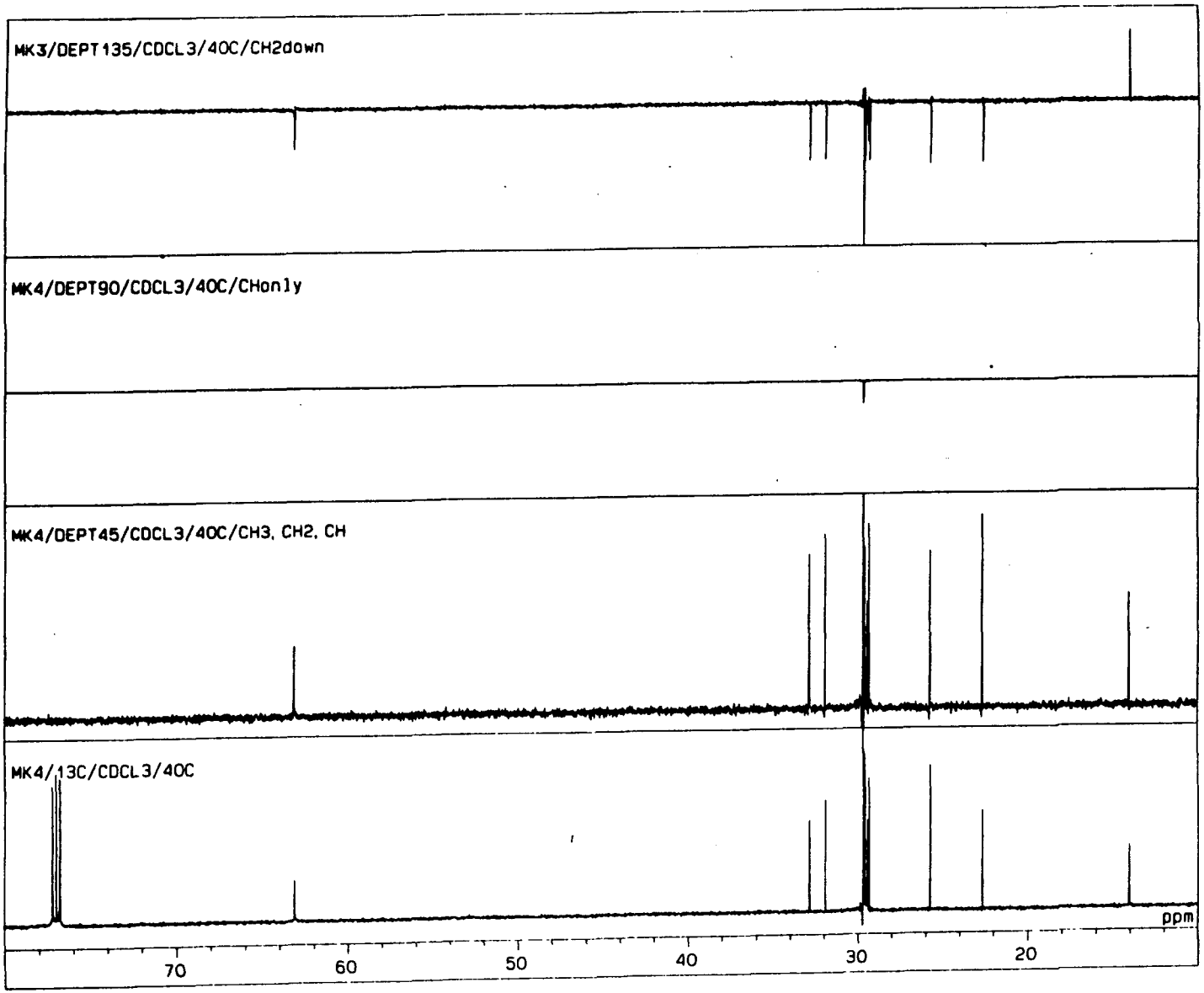
EXMOD : SINGL
IRMOD : BCM
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 2000
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23
PW1 : 9.30 usec
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 40.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 77.00 ppm
XE : 10562.06 Hz
XS : 8397.30 Hz
operator

Fig.9



17-AUG-1995 16:31:45.40

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IRMOD : BCM
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 2000
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23

PW1 : 9.30 usec

OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 40.0 c
CSPED : 13 Hz
SLVNT : CDCL3

RESOL : 2.48 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 77.00 ppm
XE : 10562.06 Hz
XS : 8397.30 Hz
operator

Fig.10

supported by DEPT experiments (150.8 MHz, $CDCl_3$) shown in Fig. 10.

MK-4 crystallized from chloroform, m.p. 235-37°C, $C_{23}H_{25}NO$ (M^+ 331), gave brown fluorescence in UV light after developing on TLC plate (silica gel.) The IR spectrum shows bands at 1640 and 1600 cm^{-1}, characteristic of unsaturation.The ^1H NMR spectrum (Fig.11) in $CDCl_3$ at 100 MHz was comparable with that of mahanimbidine[8] and shows singlets for methyl groups at δ 1.32, 1.48, 1.92 and 2.34 attributable to 8'-Me, 3'-Me, 9'-Me and 3-Me, respectively (Table II). The multiplets appearing at δ 0.20 and 1.40 were assigned to 5'-ax-H and 5'-eq-H, respectively. The benzylic proton at 1'- position appears as a broad doublet at δ 3.35. The aromatic proton at C-4 appears as a singlet at δ 7.35. Other aromatic protons absorb between δ 6.30 - 7.90 as double doublets or multiplets integrating for four protons at 5, 6, 7and 8 carbons. Although chemical and spectral data of **MK-4** (m.p. 234-37°C) are comparable with **mahanimbidine (Xb)[8]** (**=currayangine = murrazoline**)[25, 27, 34] m.p. 266°C, yet

Table II. ^1H NMR data of compound MK-4 (100 MHz, $CDCl_3$)

Assignment	Chemical shift (δ, ppm)
5'-ax-H	0.20 (m,1H)
8'-Me	1.32 (s, 3H)
5'-eq-H	1.40 (m,3H)
3'-Me	1.48 (s, 3H)
9'-Me	2.34 (s, 3H)
3-Me	2.34 (s, 3H)
1'-H	3.35 (brd, 1H)
Ar - CH_3	7.35 (s, 3H)
Ar-H	6.30-7.90 (m, 5H)

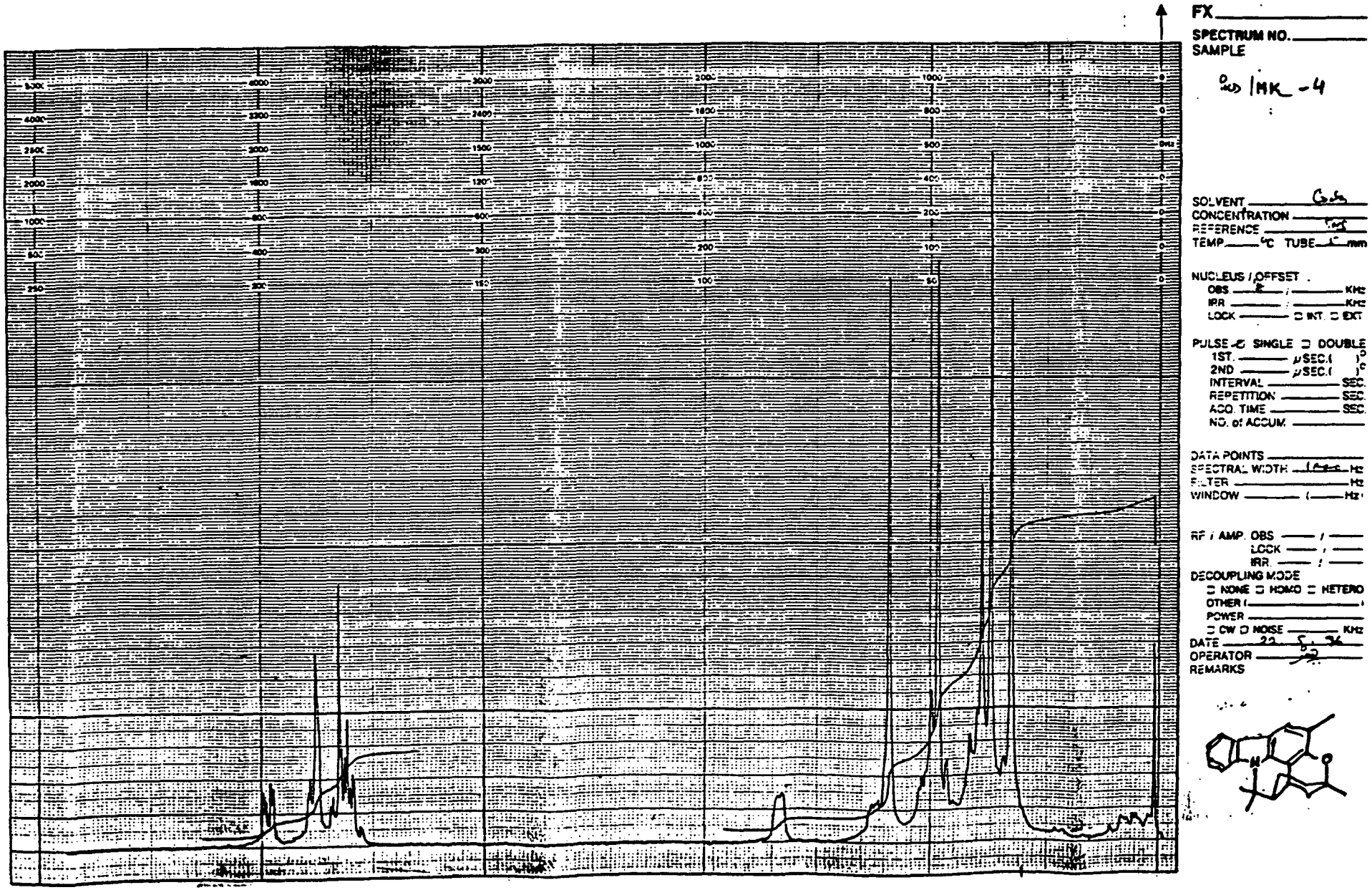
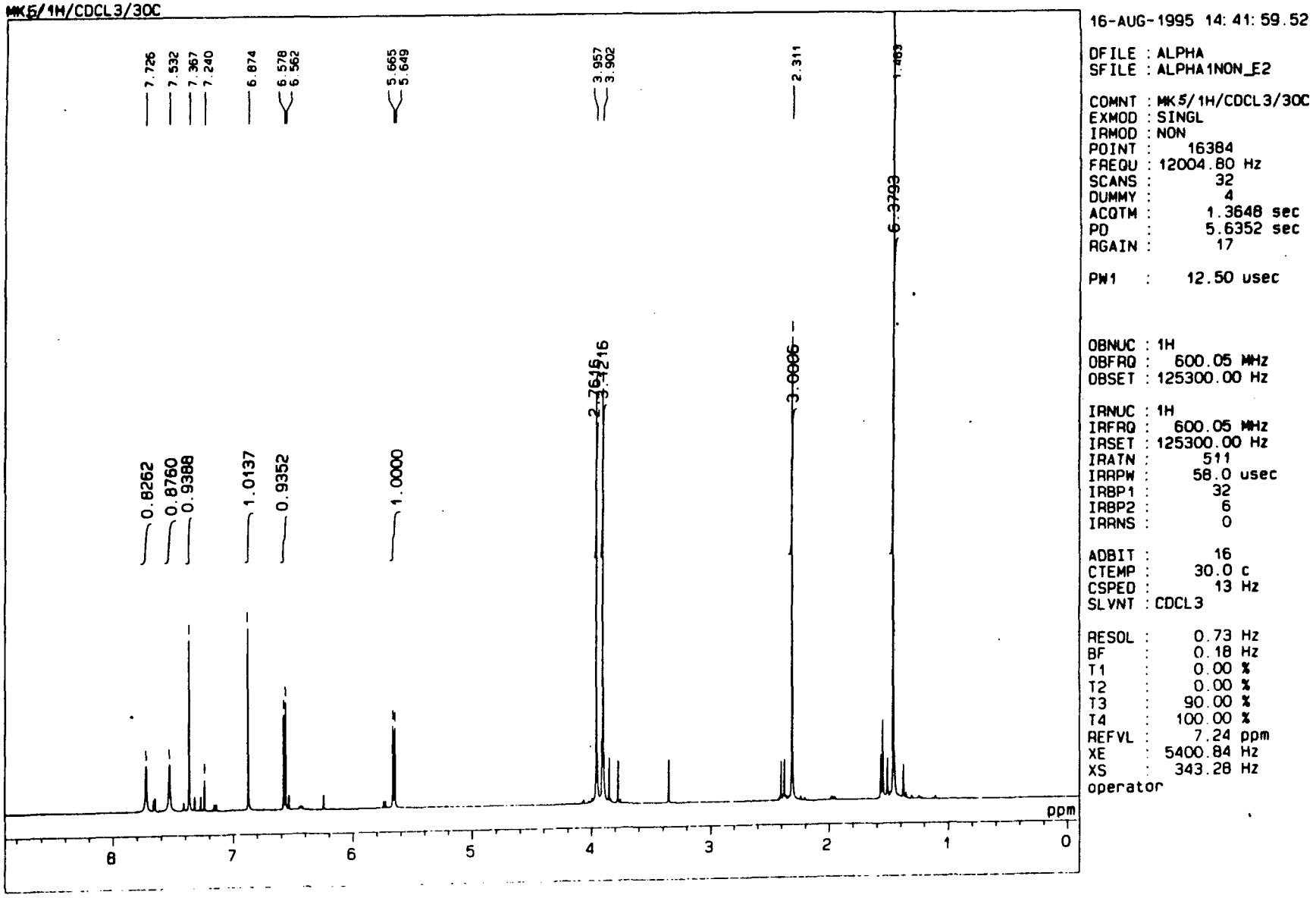

FX
SPECTRUM NO.
SAMPLE
MK-4
SOLVENT
CONCENTRATION
REFERENCE
TEMP °C TUBE mm
NUCLEUS / OFFSET
OBS / KHz
IRR / KHz
LOCK ☐ INT. ☐ EXT.
PULSE ☑ SINGLE ☐ DOUBLE
1ST. μSEC. (
2ND μSEC. (
INTERVAL SEC.
REPETITION SEC.
ACQ. TIME SEC.
NO. of ACCUM.
DATA POINTS
SPECTRAL WIDTH Hz
FILTER Hz
WINDOW (Hz
RF / AMP. OBS /
LOCK /
IRR. /
DECOUPLING MODE
☐ NONE ☐ HOMO ☐ HETERO
OTHER (
POWER
☐ CW ☐ NOISE KHz
DATE
OPERATOR
REMARKS

Fig.11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6.578
6.562
5.665
5.649
3.957
3.902
2.311
1.463
0.8262
0.8760
0.9388
1.0137
0.9352
1.0000
2.7616
3.0000
6.3793
ppm
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6
5
4
3
2
1
0
16-AUG-1995 14:41:59.52
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IRMOD : NON
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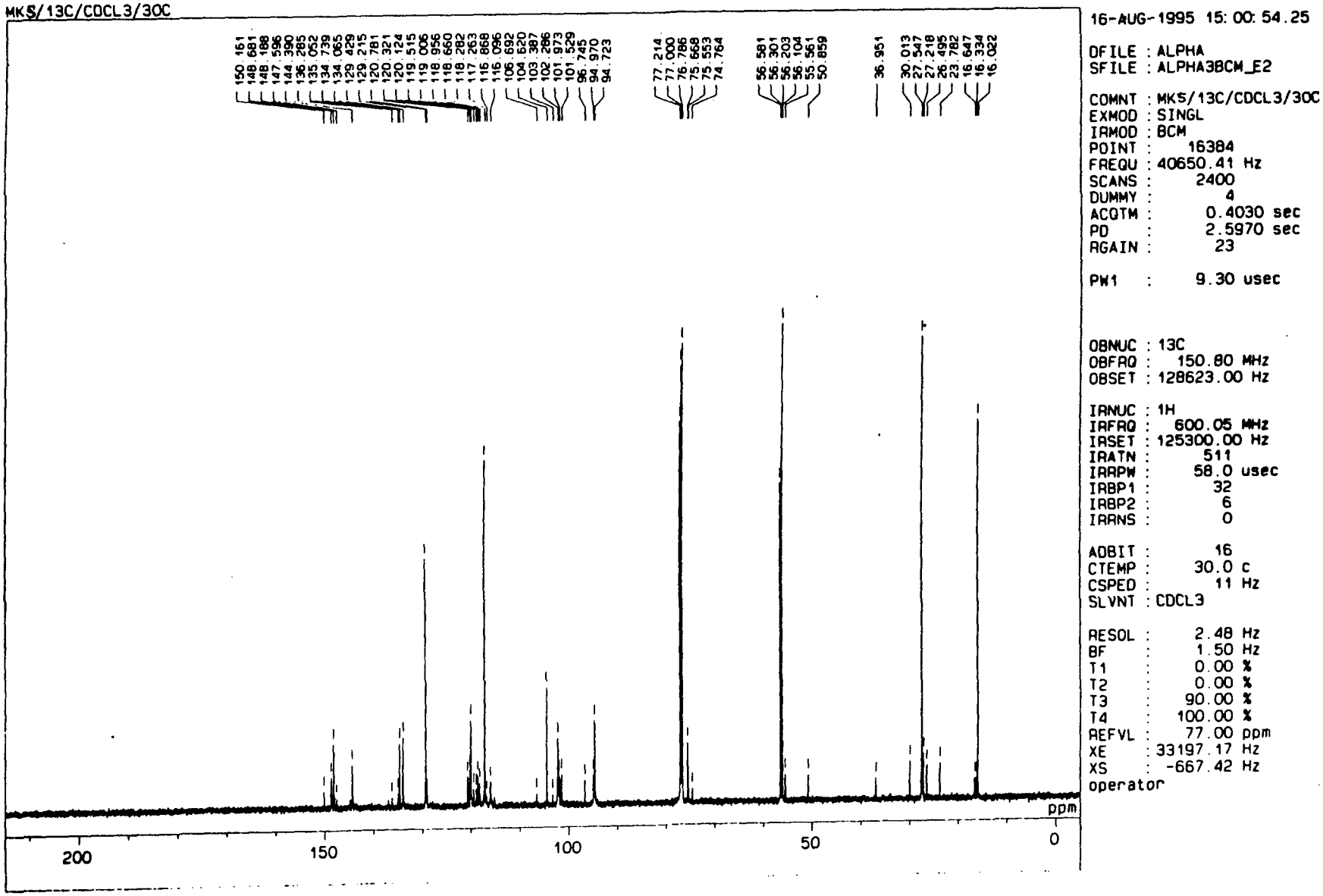
FREQU : 12004.80 Hz
SCANS : 32
DUMMY : 4
ACQTM : 1.3648 sec
PD : 5.6352 sec
RGAIN : 17
PW1 : 12.50 usec
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125300.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 0.73 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 7.24 ppm
XE : 5400.84 Hz
XS : 343.28 Hz
operator

Fig.12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148.188
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144.390
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135.052
134.739
134.065
129.429
129.215
120.781
120.321
120.124
119.515
119.006
118.956
118.660
118.282
117.263
116.868
116.096
106.692
104.620
103.387
102.286
101.973
101.529
96.745
94.970
94.723
77.214
77.000
76.786
75.668
75.553
74.764
56.581
56.301
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36.951
30.013
27.547
27.218
26.495
23.782
16.647
16.334
16.022
ppm
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150
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50
0
16-AUG-1995 15:00:54.25
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IRMOD : BCM
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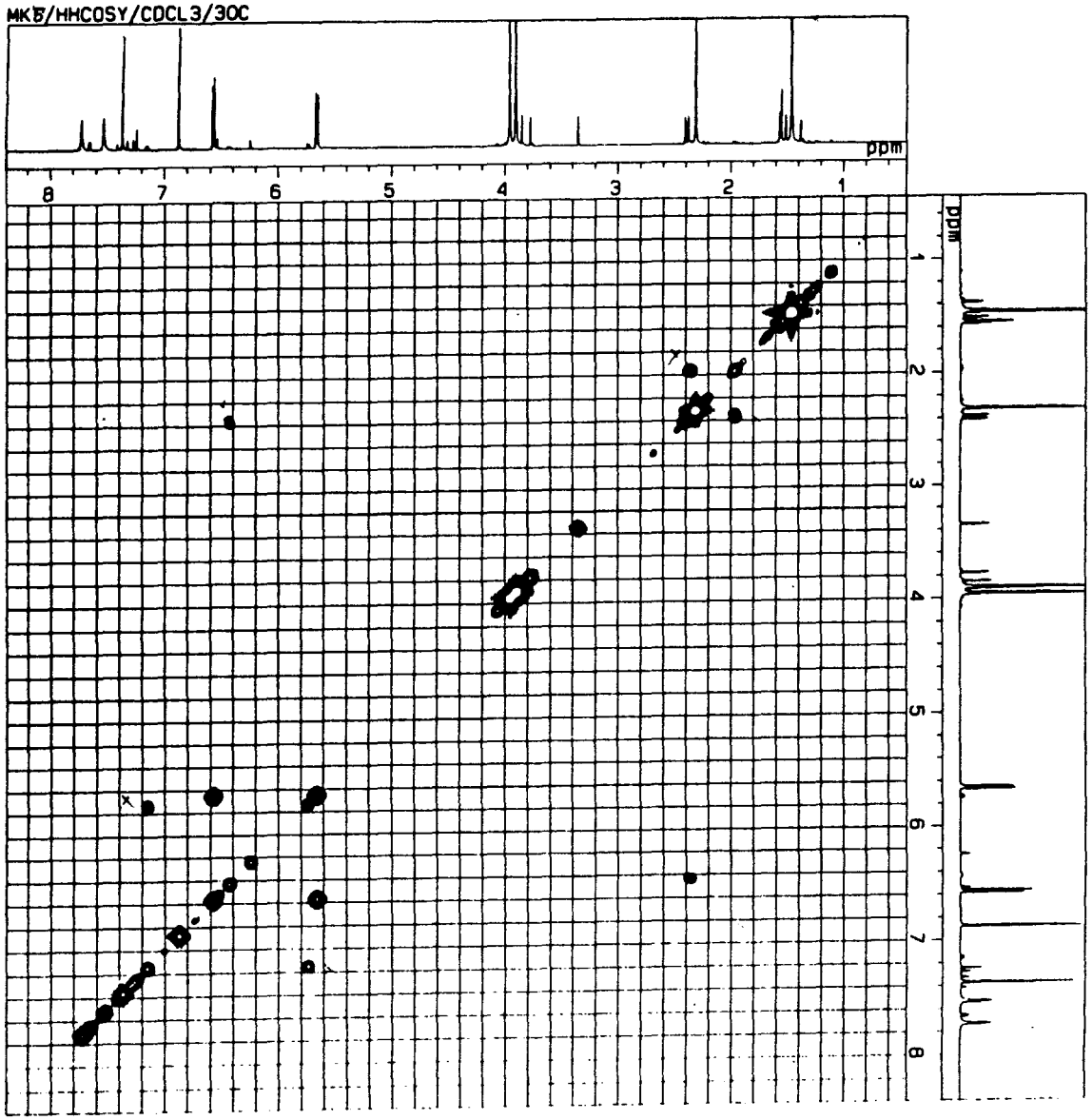
FREQU : 40650.41 Hz
SCANS : 2400
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23
PW1 : 9.30 usec
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 11 Hz
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 1.50 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 77.00 ppm
XE : 33197.17 Hz
XS : -667.42 Hz
operator

Fig.13



16-AUG-1995 14: 49: 11.69

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HR2FILE: ALPHA1NON_E2
HR1FILE: ALPHA1NON_E2

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POINT : 512
FREQU : 4768.72 Hz
SCANS : 8
DUMMY : 4
ACQTM : 0.0537 sec
PD : 0.9463 sec
AGAIN : 14

CLFRQ : 4768.72 Hz
CLPNT : 512
TOSCN : 256
CINWT : 10.00 usec
CINTV : 209.70 usec

PW1 : 12.50 usec
PW2 : 20.00 usec
PI1 : 120.0000 msec
PI2 : 1.0000 msec

OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 124966.98 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
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RESOL : 9.31 Hz
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TLINE : 16
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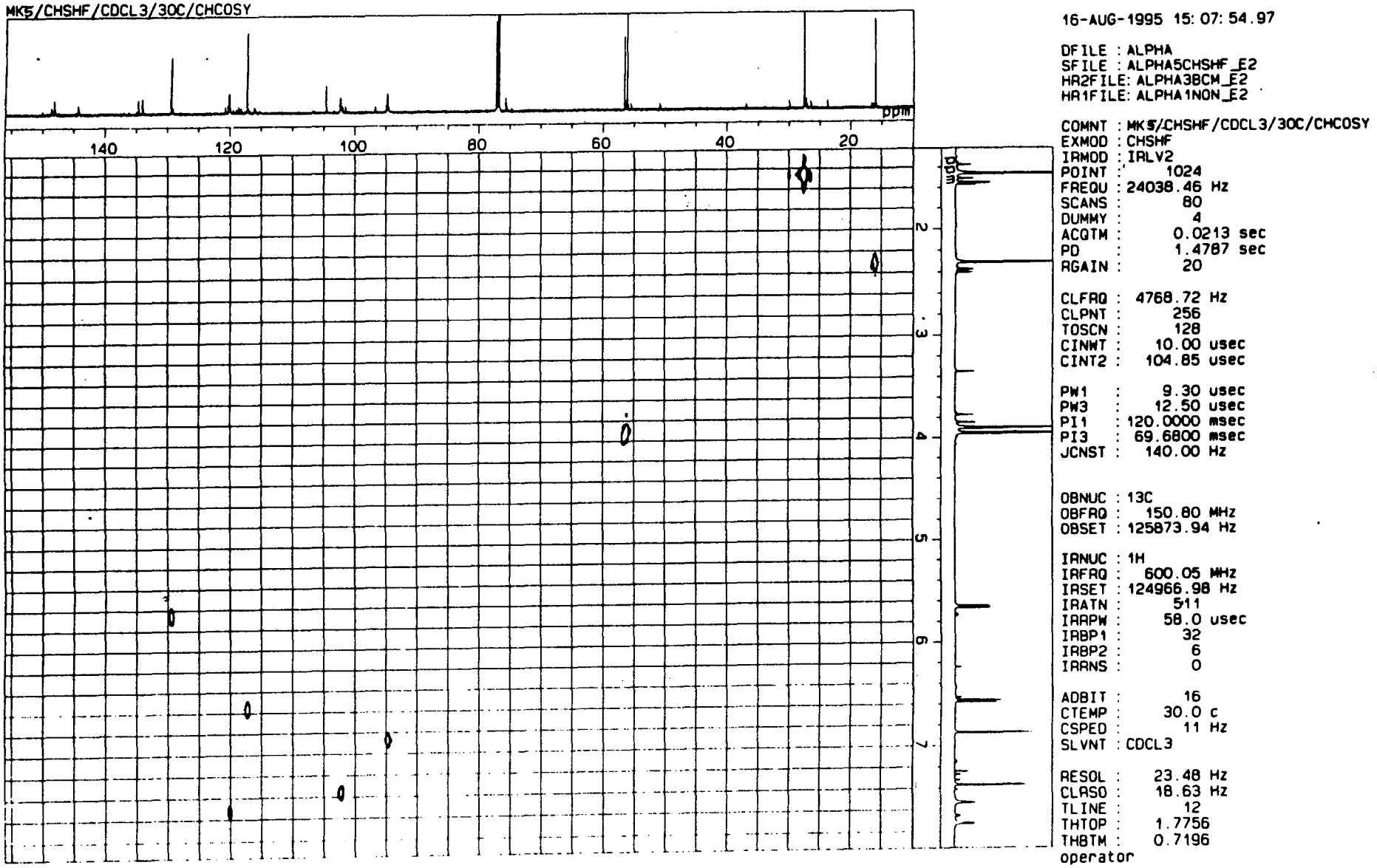
operator

Fig.14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ppm
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120
100
80
60
40
20
2
3
4
5
6
7
ppm
16-AUG-1995 15:07:54.97
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HR2FILE: ALPHA3BCM_E2
HR1FILE: ALPHA1NON_E2
COMNT : MK5/CHSHF/CDCL3/30C/CHCOSY
EXMOD : CHSHF
IRMOD : IRLV2
POINT : 1024
FREQU : 24038.46 Hz
SCANS : 80
DUMMY : 4
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PD : 1.4787 sec
RGAIN : 20
CLFRQ : 4768.72 Hz
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TOSCN : 128
CINWT : 10.00 usec
CINT2 : 104.85 usec
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PW3 : 12.50 usec
PI1 : 120.0000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 125873.94 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 124966.98 Hz
IRATN : 511
IRPW : 58.0 usec
IRBP1 : 32
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CSPED : 11 Hz
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RESOL : 23.48 Hz
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THTOP : 1.7756
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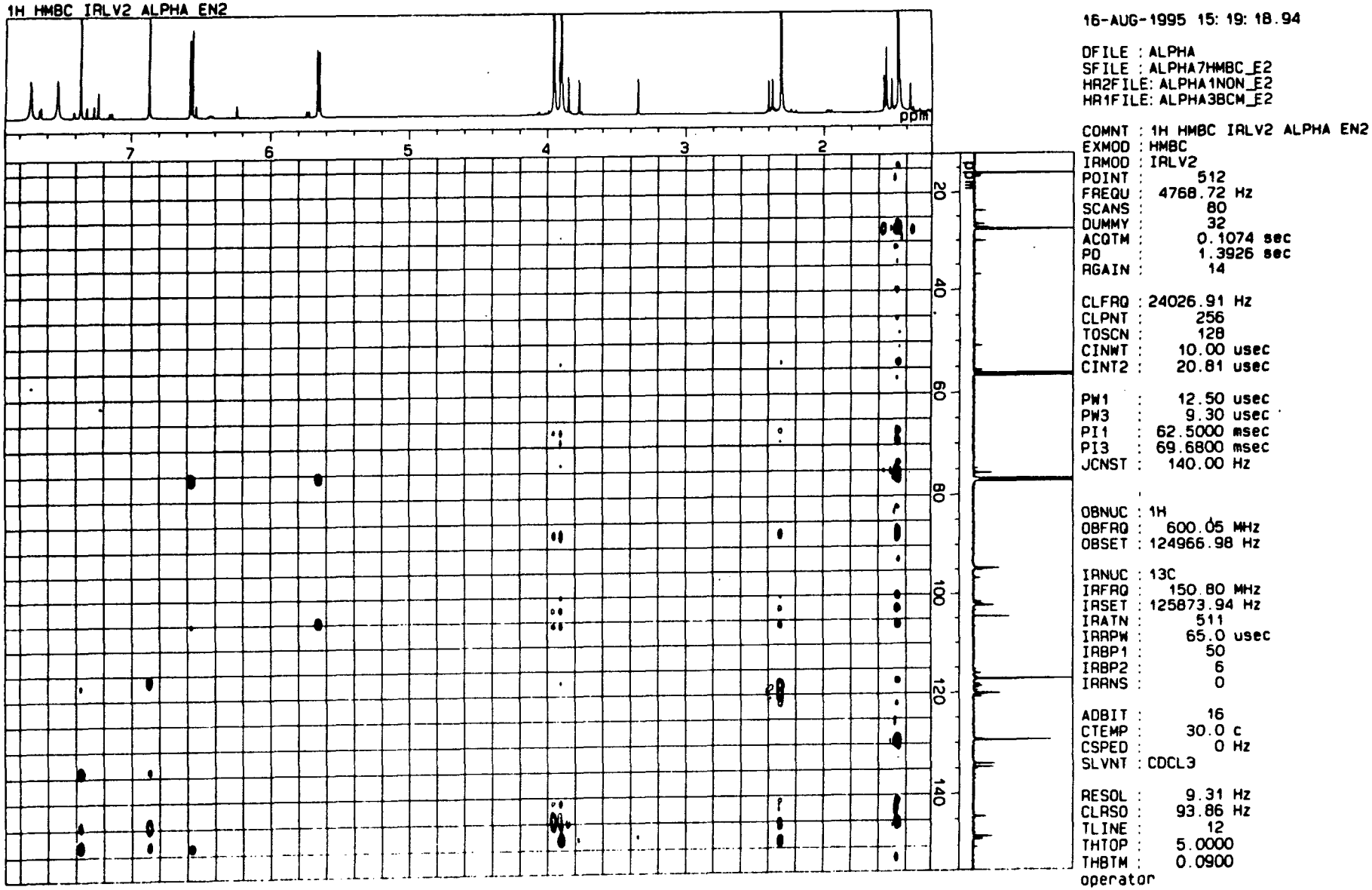
operator

Fig.15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ppm
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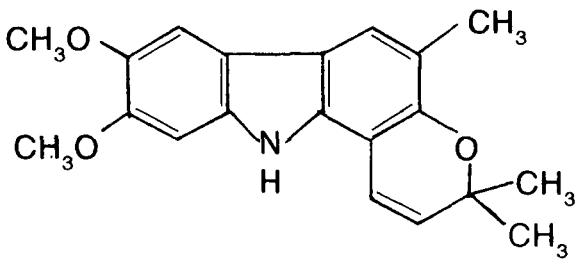
6
5
4
3
2
20
40
60
80
100
120
140
ppm
16-AUG-1995 15: 19: 18.94
DFILE : ALPHA
SFILE : ALPHA7HMBC_E2
HR2FILE: ALPHA1NON_E2
HR1FILE: ALPHA3BCM_E2
COMNT : 1H HMBC IRLV2 ALPHA EN2
EXMOD : HMBC
IRMOD : IRLV2
POINT : 512
FREQU : 4768.72 Hz
SCANS : 80
DUMMY : 32
ACQTM : 0.1074 sec
PD : 1.3926 sec
RGAIN : 14
CLFRQ : 24026.91 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 20.81 usec
PW1 : 12.50 usec
PW3 : 9.30 usec
PI1 : 62.5000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 124966.98 Hz
IRNUC : 13C
IRFRQ : 150.80 MHz
IRSET : 125873.94 Hz
IRATN : 511
IRRPW : 65.0 usec
IRBP1 : 50
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 30.0 c
CSPED : 0 Hz
SLVNT : CDCL3
RESOL : 9.31 Hz
CLRSO : 93.86 Hz
TLINE : 12
THTOP : 5.0000
THBTM : 0.0900
operator

Fig.16

its structural assignment is not conclusive due to large difference in melting point. Further studies are required to settle the stereochemistry as well as structure.

The benzene fraction on column chromatography (silica gel), eluting the column with petrol - benzene (1:1) and benzene - ethyl acetate (8:2) gave **Mk-5** and **MK-6**.

MK-5 was obtained as light yellowish green crystals from benzene - alcohol. It melts at 225°C and shows brown fluorescence in UV light after development on TLC plate (silica gel). The IR spectrum shows bands at 3400 (N-H), 1640, 1620 and 1600 cm^{-1} (unsaturation and aromatic system). In the mass spectrum molecular ion appeared at m/z 323 and base peak at m/z 308. The ^{1}H NMR spectrum (Table III and Fig. 12) was comparable with that of **koenidine (IVb)**[17] **(=Koenigicine = Koenimbidine)**[19-21], m.p. 224-25°C. The structure was confirmed by ^{13}C NMR (Table III and Fig. 13), DEPT experiments, HHCOSY (Fig. 14), CHCOSY (Fig.15), COLOC and HMBC (Fig. 16) studies which was not done earlier.



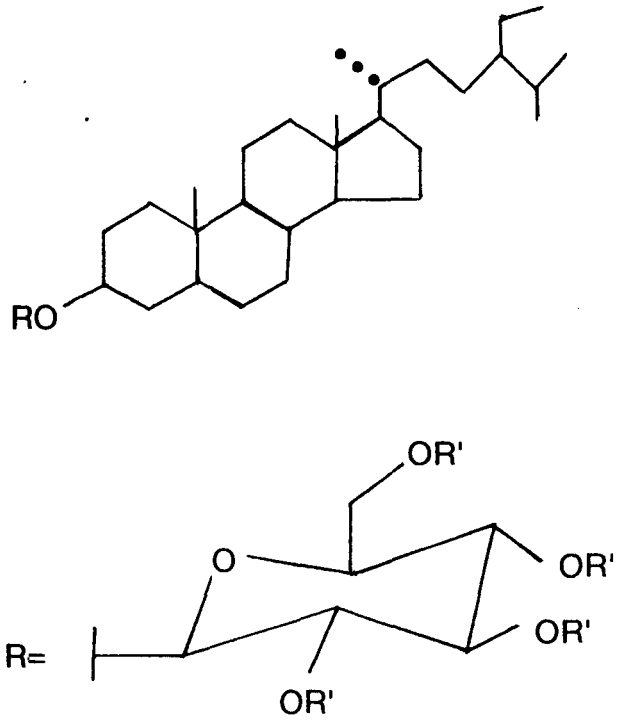
IV b

MK-6 was obtained as light green granules from pyridine - benzene - methanol (1:11:10). It melts at 288 - 90°C (lit. 290°C)[45] and was detected by iodine and perchloric acid on TLC plate (silica gel.). In the infra red spectrum it

Table III. ^{1}H NMR and ^{13}C NMR data of MK-5 (IVb)

Assignment	Chemical shift (δ , ppm)	
C/H	^{13}C NMR (150.8 MHz, $CDCl_3$)	^{1}H NMR (600 MHz, $CDCl_3$)
1	104.620	-
2	148.681	-
3	118.282	-
4	120.124	7.532 (s)
4a	124.448	-
4b	116.868	-
5	102.286	7.367 (s)
6	148.188	-
7	144.390	-
8	94.723	6.874 (s)
8a	134.410	-
9	-	7.726 (s)
9a	135.759	-
3-Me	16.022	2.311 (s)
6-0Me	56.301	3.902 (s)
7-oMe	56.581	3.957 (s)
2'	75.668	-
3'	129.429	5.657 (d, J=10Hz)
4'	117.263	6.570 (d, J=10Hz)
2'-Me_2	27.547	1.463 (s)

shows strong absorption at 3350 cm^{-1} indicating the presence of -OH group. Its melting point and IR spectrum indicated it to be sterol glycoside. The structure was assigned as **sitosterol - 3 - 0 - β - D - glucoside (XIVa)** from IR and ^1H NMR (100 MHz, $CDCl_3$) given in Fig. 17 and Table IV of its acetylated derivative (**XIVb**). The occurrence of sitosterol - 3 - O - β - D - glycoside from **M. Koenigii** has not been reported so far.



XIV

a R' = H

b R' = Ac

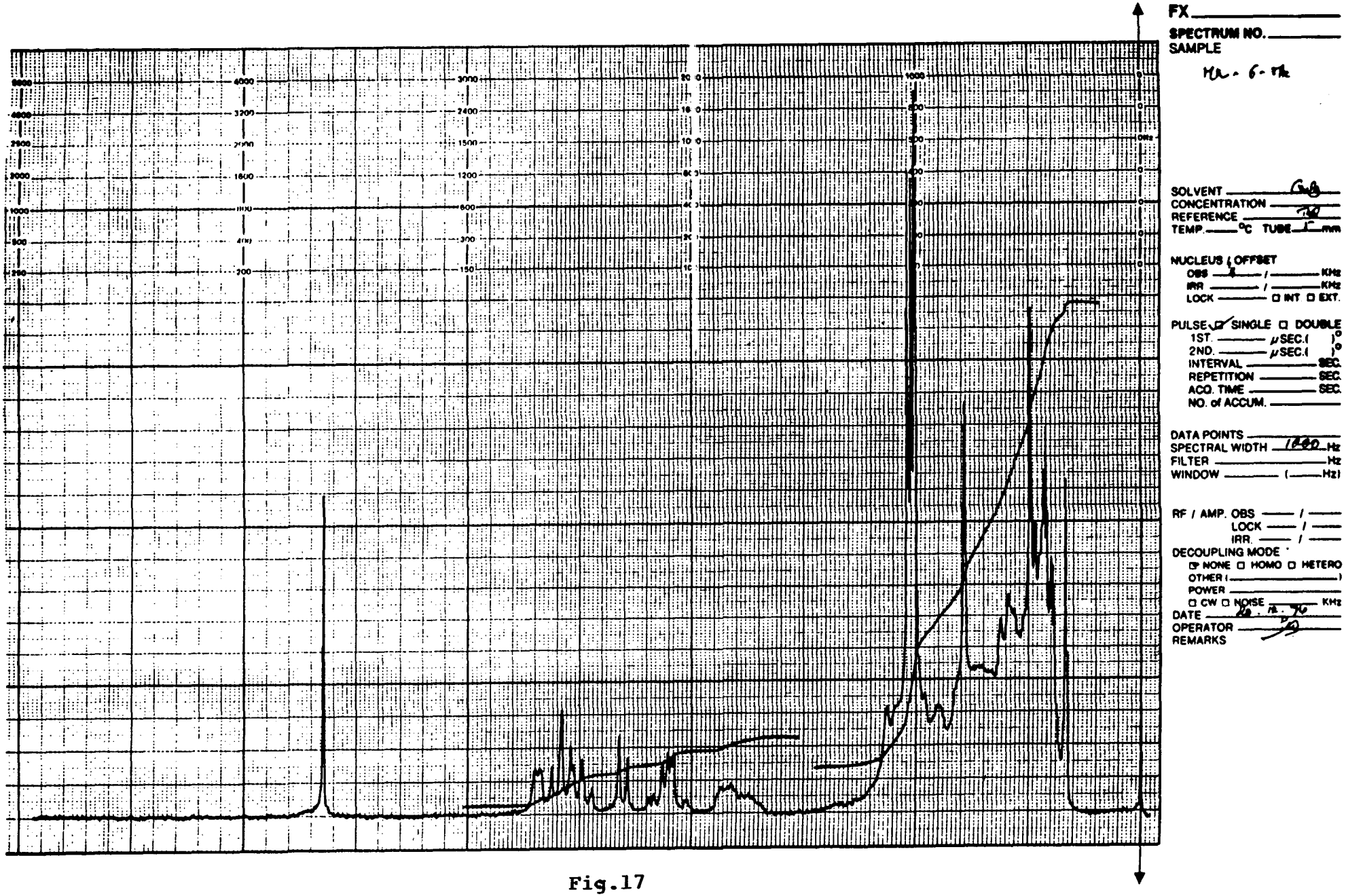

FX
SPECTRUM NO.
SAMPLE
Mr. 6-Me
SOLVENT
CONCENTRATION
REFERENCE
TEMP. °C TUBE mm
NUCLEUS / OFFSET
OBS / KHz
IRR / KHz
LOCK ☐ INT ☐ EXT.
PULSE ☑ SINGLE ☐ DOUBLE
1ST. μSEC.( )°
2ND. μSEC.( )°
INTERVAL SEC.
REPETITION SEC.
ACQ. TIME SEC.
NO. of ACCUM.
DATA POINTS
SPECTRAL WIDTH 1000 Hz
FILTER Hz
WINDOW (Hz)
RF / AMP. OBS /
LOCK /
IRR /
DECOUPLING MODE
☑ NONE ☐ HOMO ☐ HETERO
OTHER ( )
POWER
☐ CW ☐ NOISE KHz
DATE
OPERATOR
REMARKS

Fig.17

Table IV. ^1H NMR data of MK-6 tetraacetate (100 MHz, $CDCl_3$)

Assignment	Chemical shift (δ , ppm)
18 - Me	0.68 (s, 3H)
28 - Me	0.76 (d, J=6.8 Hz, 3H)
26, 27 -Me	0.84 (d, J=6.5 Hz, 6H)
21-Me	0.90 (d, J=6.5 Hz, 3H)
19-Me	1.00 (s, 3H)
-OCO Me	1.88-2.32 (s, 12H)
$-CH_2O-$	3.22 - 3.80 (m, 8H)
=CH	5.40 (m, 1H)
-CH2, -CH	1.12 - 1.60 (cyclic & side (chain)

EXPERIMENTAL

The dried leaves (1.5 kg) of *M. koenigii* were extracted with methanol. Repeated extractions were made until the solution become colourless. The methanol extracts were mixed and dried under reduced pressure. A dark green gummy mass (100g) was obtained which was treated with petrol, benzene, ethyl acetate and acetone several times till solvent become colourless in each case.

The petrol fraction (40g) was adsorbed on silica gel (30g) and transferred to column of silica gel (150g) set with petroleum ether (60-80°C). The elution of column with petrol - benzene (1:1) gave crude **MK-1** which on dissolution in benzene - chloroform, crystallization and recrystallization gave pure **MK-1.**

MK-1: Crystalline compound (400 mg), crystallized in benzene which melts at 205°C and dissolves readily in chloroform. Rf=0.2958 (silica gel, petrol- benzene, 4:6) , in UV light gave brown fluorescent, adsorb iodine, developed on charring with perchloric acid and with alcoholic $FeCl_3$ gave yellowish green colouration.

UV, IR, MS and ^{1}H NMR data of MK-1.

UV λ_{max}^{EtOH} nm (log ε) : 360 (4.20), 340 (4.24) , 300 (4.62), 240 (4.69), 230 (4.68).

IR ν_{max}^{KBr} cm^{-1} : 3400, 2950, 2850, 1640, 1610, 1580, 1490, 1450, 1420, 1400, 1380, 1360, 1330, 1290, 1260, 1240, 1200, 1170, 1130, 1120, 1100, 1050, 1020, 890, 870, 840, 800, 770, 760, 720, 680.

MS m/z (rel. int.) : 293 $[M]^+$ (40), 278 [M-15] (100) aromatization of chromene ring by loss of Me group, 262 (10), 235 (22), 191 (10), 139 (16), 117 (10), 77(10), 53 (18).

^{1}H NMR (600 MHz) : δ 1.485 (s, 6H, gem dimethyl protons), 2.340, (s, 3H, 3-Me), 3.903 (s, 3H, Ar-OCH_3), 5.640 (d, J=10 Hz, 3'-H), 6.536 (d, J=10Hz, 4'-H), 6.940 (dd, J=8.4, 2.2 Hz, 7-H), 7.214 (d, J=8.4 Hz, 8-H), 7.421 (d, J=2.2 Hz, 5-H), 7.627 (s, IH, 4-H), 7.686 (br s, IH, N-H).

^{13}C NMR data is given in discussion (Table I).

From UV, MS, IR, ^{1}H NMR and ^{13}C NMR spectral data the compound was found to be **koenimbine = koenimbin**.

Further elution of column with petrol - benzene (1:1) and crystallization in ethanol - chloroform (8:2) crude **MK-2** was obtained.

MK-2 : Crude MK-2 on crystallization in benzene gave colourless crystals (180 mg) of Mk-2 (pure), readily dissolved in chloroform and having melting point 139-41^{0}C. Rf=0.7384 (silica gel, benzene - acetone, 9:1) . UV inactive but appeared on absorption with iodine and charring with perchloric acid.

IR, MS and ^{1}H NMR data of MK-2.

IRν_{max}^{KBr} cm $^{-1}$: 3400, 3250, 2900, 2850, 1640, 1370, 1050, 950, 880.

MS m/z (rel. int.) : 414 $[M]^+$ (100), 399 (25), 396 (30), 381 (18), 273 (50), 271 (44), 255 (60), 231 (30), 213 (50).

^{1}H NMR (100 MHz) : δ 0.68 (s, 3H, 18-Me), 0.75 (d, J=6.8 Hz, 3H, 28 -Me), 0.84 (d, J=6.5 Hz, 6H, 26, 27-Me), 0.92 (d, J=6.5 Hz, 3H, 21 -Me), 1.01 (s, 3H, 19-Me), 1.07 - 2.34 (-CH_2 and -CH protons of cyclic and side chain), 3.52 (m, 1H, 3-ax-H), 5.15 (m, IH, -OH), 5.39 (m, 1H, olefinic proton).

From IR, MS, ^{1}H NMR spectral data and on direct comparison with authentic sample the compound was found to be **β- sitosterol**.

Elution of petrol fraction column with benzene, applying preparative thin

layer chromatography (PTLC) and on crystallization **MK-3** was isolated.

MK-3 : Colourless granular compound (120 mg), soluble in chloroform, crystallized in alcohol - benzene (7:3) having melting point 94-5°C. Rf= 0.6484 (silica gel, benzene, PTLC), UV inactive, absorb iodine and developed on charring with perchloric acid.

Spectral data of MK-3.

IR υ_{max}^{KBr} cm^{-1} : 3300, 2900, 2820, 2300, 1460, 1420, 1370, 1330, 1060, 1010, 760, 720.

MS m/z (rel. int.) : 476 $[M]^+$ (3.5), 462 (1.2), 448 (7.5), 434 (1.4), 420 (5), 406 (1), 181 (2), 167 (3), 153 (4), 139 (10), 125 (19), 111 (36), 97 (66), 83 (69), 69 (56), 57 (100).

^1H NMR and ^{13}C NMR is given in discussion.

From IR, MS, ^1H NMR and ^{13}C NMR the compound was found to be a **long chain alcohol**.

Further elution of column with benzene, crystallization and recrystallization in benzene **MK-4** was isolated.

MK-4 : Colourless, crystalline compound (13 mg) which dissolves readily in chloroform and melts at 235 - 37°C. Rf = 0.8238 (silica gel, petrol - benzene, 4:6), in UV light gave brown fluorescent, absorb iodine and developed on charring with perchloric acid.

Spectral data of MK-4 :

UV λ_{max}^{EtOH}(log ε) 241 (4.62), 257 (4.37), 307 (4.20), 355 (3.51)

IR ν_{max}^{KBr} cm^{-1} : 2950, 2900, 1640, 1600, 1490, 1450, 1370, 1350, 1330, 1300,

1250, 1220, 1200, 1180, 1150, 1120,1080, 1060, 990, 850, 740.

MS 70 eV, m/z : 331 $[M]^+$, 316 [base peak], 248.

^{1}H NMR (100 MHz) : δ 0.20 (m, 1H, 5'-ax-H), 1.32 (s, 3H, 8'-Me), 1.40 (m, 1H,5'-eq-H), 1.48 (s, 3H,3'-Me), 1.92 (s, 3H, 9'-Me), 2.34 (s, 3H, 3-Me), 3.35 (br d, 1H, benzylic proton at 1'), 7.35 (s, 3H, Ph - CH_3), 6.30 - 7-90 (m, 5H, Ar-H).

From UV, IR, MS and ^{1}H NMR spectral data the compound was found to be **mahanimbidine = currayangine = murrazoline**.

The benzene fraction (18g) adsorbed on silica gel (15g) and transferred to the silica gel column set with petroleum ether (60-80°C). Elution of column with petrol - benzene (1:1) and crystallization in alcohol - benzene (8:2) **MK-5** was isolated.

MK-5 : Light yellowish green crystals (600 mg) melting point 225°C and soluble in chloroform. Rf=0.3584 (SiO_2, benzene), in UV light gave brown fluorescent, adsorb iodine, developed on charring with perchloric acid and with alchoholic $FeCl_3$ gave green colouration.

Spectral data of MK-5 .

UV λ_{max}^{EtOH} (log ε) : 235 (4.56), 240 (4.57) , 288 (4.36), 297 (4.53), 336 (3.97).

IR υ_{max}^{KBr} cm^{-1} : 3400, 2900, 2800, 1640, 1620, 1600, 1585, 1490, 1470, 1455, 1440, 1420, 1400,1370, 1355, 1340, 1290, 1270, 1240, 1200, 1185, 1170, 1150, 1120, 1110, 1050, 1030, 990, 970, 940, 920, 860, 820, 760, 740, 720, 670.

MS m/s (rel. int.) : 323 $[M]^+$ (60), 308 [M-15] (100) aromatization of chromene ring by loss of methyl group, 293 [M-30] (20) further loss of methyl group,

264 (10), 250 (16), 154 (14), 133 (10), 71 (16).

^{1}H NMR (600 MHz) : δ 1.463 (s, 6H, 2'-Me_2), 2.311 (s, 3H, 3-Me), 3.902 (s, 3H, 6-OMe), 3.957 (s, 3H, 7-OMe), 5.657(d, J=10Hz, 1H, 3'-H), 6.570 (d, J=10Hz, 1H, 4'-H), 6.874 (s, 1H, 8-H), 7.367 (s, 1H, 5-H), 7.532 (s, 1H, 4-H).

^{13}C NMR is given in discussion (Table III). From UV, IR, MS, ^{1}H NMR and ^{13}C NMR the compound was found to be **koenimbidine = koenigicine = koenidine.**

Further elution of column with benzene - ethyl acetate (8:2) and on crystallization in pyridine - benzene - methanol (1:11:10) **MK-6** was isolated.

MK-6 : Light green granules (105g), soluble in pyridine and boiling methanol, melt at 290°C. Rf= 0.6421 (SiO_2, ethyl acetate - methanol, 8:2), UV inactive, adsorb iodine and developed on charring with perchloric acid.

IR data of MK-6 :

IR υ_{max}^{KBr} cm^{-1} : 3350, 2950, 2850, 1460, 1430, 1380, 1370, 1160, 1110, 1070, 1010, 950, 920, 880.

Acetylation of MK-6: A tetra acetate (m.p. 158°C) was prepared by treatment of **MK-6** with Ac_2O and pyridine. Rf=0.900 (SiO_2; benzene - acetone, 7:1), developed by adsorption of iodine and charring with perchloric acid.

IR υ_{max}^{KBr} cm^{-1} : 2900, 2850, 1750, 1740, 1460, 1430, 1370, 1210, 1150,1030, 900

^{1}H NMR (100 MHz) : δ 0.68 (s, 3H, 18-Me), 0.76 (d, J=6.8 Hz, 3H, 28-Me), 0.84 (d, J=6.5 Hz, 6H, 26, 27-Me), 0.90 (d, J=6.5 Hz, 3H, 21-Me), 1.00 (s, 3H, 19-Me), 1.88 - 2.32 (s, 12H, - OCO CH_3), 3.22 - 3.80 (m, 8H, -CH_2O-), 5.40 (m, 1H, =CH), 1.12 -1.60 (-CH_2, - CH protons of cyclic and side chain).

From IR of **MK-6,** IR and ^{1}H NMR of its tetraacetate derivative the compound was found to be **sitosterol - 3 - O - β - D - glucoside.**

REFERENCES

1. Chowdhury, B.K. and Chakraborty, D.P. *Chem. Ind.* **1969**, 17, 549.

2. Chowdhury, B.K. and Chakraborty, D.P. *Phytochemistry,* **1971**, 10 (8), 1967.

3. Bhattacharyya, P. and Chakraborty, A. *Phytochemistry*, **1984**, 23 (2), 471.

4. Bhattacharyya, P. ; Maiti, A.K. ; Basu, K. and Chowdhury, B.K. *Phytochemistry* **1994**, 34 (4), 1085.

5. Fiebig, M. ; Pezzuto, J.M.; Soejarto, D.D. and Kinghorn, A.D. *Phytochemistry* **1985**, 24 (12), 3041.

6. Bhattacharyya, P. and Chowdhury, B.K. *Indian J. Chem. Sect. B* **1985**, 24 B (4), 452.

7. Gupta, G.L. and Nigam, S.S.*Planta Med.* **1971,** 19 (1), 83.

8. Rama Rao, A.V. ; Bhide, K. S. and Majumdar, R.B. *Chem. Ind.* **1980,** 17, 697.

9. Chakraborty, D.P. ; Roy, S. and Guha, R. *J. Indian Chem. Soc.* **1978**, 55 (11), 1114.

10. Roy, S. ; Bhattacharya, L. and Chakraborty, D. P. *J. Indian Chem. Soc.* **1982**, 59 (11), 1369.

11. Chakraborty, D.P. ; Bhattacharyya, P. ; Roy, S.; Bhattacharya, S.P. and Biswas, A.K. *Phytochemistry* **1978**, 17 (4), 834.

12. Bhattacharyya, P.; Sagar Sona, Y. and Chowdhury, B.K. *Chem. Ind.***1986**, 7, 246.

13. Adesina, S.K. ; Olatunji, O.A. ; Bergenthal, D. and Reisch, J. *Pharmazie* **1988**, 43 (3), 221.

14. Ito, C. ; Thoyama, Y. ; Omura, M. ; Kajiura, I. and Furukawa, H. *Chem. Pharm. Bull.* **1993**. 41 (2), 2096.

15. Reisch, J. ; Adebajo,A.C. ; Kumar, V. and Aladesanmi, A. J. *Phytochemistry* **1994**, 36 (4), 1073.

16. Reisch, J. ; Goj. O. ; Wickramasinghe, A.; Herath, H.M.T. and Henkel,G. *Phytochemistry.* **1992**, 31 (8), 2877.

17. Joshi, B.S. ; Kamat, V.N. and Gawad, D.H. *Tetrahedron* **1970**, 26 (6), 1475.

18. Chakraborty, D.P. and Das, K.C. *Chem. Commun.* **1968**, 16, 967.

19. Narasimhan, N.S.; Paradkar, M.V. ; Chitguppi, V.P. and Kelkar, S.L. *Indian J. Chem.* **1975**, 13 (10), 998.

20. Kureel, S.P. ; Kapil, R.S. and Popli, S.P. *Experientia* **1969**, 25 (8), 790.

21. Narasimhan, N.S. ; Paradkar, M. V. and Kelkar, S.L. *Indian J Chem.* **1970**, 8 (5), 473.

22. Dutta, N.L. and Quasim, C. *Indian J. Chem.* **1969**, 7 (3), 307.

23. Narasimhan, N.S. ; Paradkar, M.V. and Chitguppi, V.P. *Tetrahedron Lett.* **1968**, 53, 5501.

24. Mukherjee,M. ; Mukherjee, S.; Shaw, A.K. and Ganguly, S.N. *Phytochemistry* **1983**, 22 (10), 2328.

25. Dutta, N.L.; Quasim, C. and Wadia, M.S. *Indian* J. *Chem.* **1969**, 7 (10)

1061.

26. Roy, S. and Chakraborty, D.P. *Phytochemistry* **1974**, 13 (12), 2893.

27. Kureel, S.P. ; Kapil, R.S. and Popli, S.P. *Tetrahedron Lett.* **1969**,44, 3857.

28. Chakraborty, D.P. ; Das, K.C. and Bose, P. K. *Sci. Cult.* **1966**, 32(2) 83.

29. Kureel, S.P. ; Kapil, R.S. and Popli, S. *Chem. Ind.* **1970**. 29, 958.

30. Atta - ur - Rahman ; Zaidi,R. and Firdous, S. *Fitoterapia* **1988**, 59 (6), 494.

31. Roy, S. ; Gosh, S. and Chakraborty, D.P. *Chem. Ind.* **1979**, 19, 669.

32. Chakrabarty, D.P. ; Islam, A. ; Basak, S.P. and Das, R. *Chem. Ind.* **1970**, 18, 593.

33. Chakraborty, D.P. ; Ganguly, S.N. ; Maji, P.N. ; Mitra, A.R. ; Das, K.C. and Weinstein, B. *Chem.Ind.* **1973**, 7, 322.

34. Narsimhan, N.S. and Kelkar, S. L. *Indian J. Chem. Sect. B* **1976**, 14B (6), 430.

35. Das, K.C. ; Chakraborty, D.P. and Bose, P. K. *Experientia* **1965**, 21 (6), 340.

36. Bhattacharya, L.; Roy, S. and Chakraborty, D.P. *Phytochemistry* **1982**, 21 (9), 2432.

37. Bhattacharya, L.; Chatterjee, S.K. ; Roy, S. and Chakraborty, D.P. *J. Indian Chem.Soc.* **1989**, 66 (2), 140.

38. Bordner, J. ; Chakraborty, D.P.; Chowdhury, B.K. ; Ganguly, S.N. ; Das, K.C. and Weinstein, B. *Experientia* **1972**, 28, 1406.

39. Penfold, A.R. and Simonsen, J.L. *J. Proc. Roy. Soc.* , N.S.Wales **1925**, 59, 146.

40. Wong, K.C. and Tie, D.Y. *J. Essent. Oil Res.* **1993**, 5 (4), 371.

41. MacLeod, A.J. and Pieris, N.M. *Phytochemistry* **1982**, 27(7), 1653.

42. Reisch, J.; Adebajo, A.C.; Aladesanmi, A.J.; Adesina, K.S.; Bergenthal, D. and Mere,U. *Planta Med.* **1994**,60 (3), 295.

43. Bhattacharyya, P. and Chakraborty, A. *J. Indian Chem. Soc.* **1984**, 61 (7), 650.

44. Jain, R.K. and Srivastava, S.D. *Proc. Natl. Acad. Sci. Sect. A.* **1992**, 62(1), 5.

45. Chaurasia, N. and Wichtl, M. *J. Nat. Prod.* **1987**, 50 (5), 881-85.

CHAPTER - III

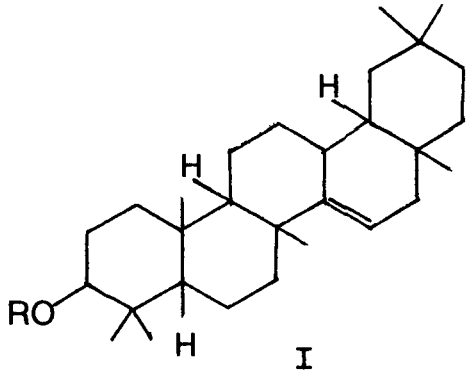
THE CONSTITUENTS OF THE ROOTS OF *GERBERA LANUGINOSA (COMPOSITAE).*

INTRODUCTION

Plants belonging to the genus **Gerbera** (Fam.: Compositae, Sub Fam. Asteraceae) have been reported to elaborate several coumarin derivatives[1-5], cyanogenic glucosides[6], sterol glycosides[4] and terpenoids[3,7], some of which exhibit antibacterial property [8,9]. **Gerbera lanuginosa** Benth. grows in India inthe Western Himalayas at altitudes of 4000 to 9500 feet. The white cotton like coating found on the under surface of the leaves is used for straunching wounds[10].

Previously Isolated Constituents:

TERPENOIDS : Taraxerol (**Ia**)[7], taraxeryl acetate (**Ib**)[7] and β-sitosterol - β - D - glucoside[4] were isolated from the whole plant of *Gerbera lanuginosa.*

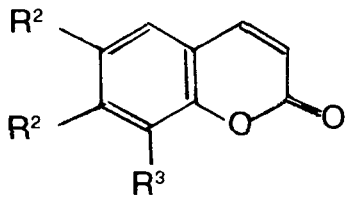


I

a R=H

b R=Ac

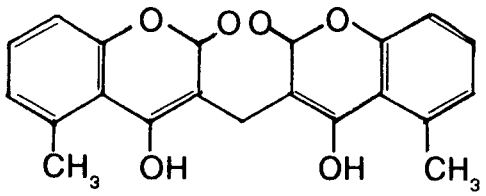
COUMARINS : Daphnetic - 7- methyl ether (**IIa**)[5], scopoletin (**IIb**)[5] and gerberinol (**III**)[4] were isolated from the whole plant of *G. lanuginosa.*



II

a R1=H, R^2=OCH^3, R^3=OH

b R1=OCH_3, R^2=OH, R^3=H



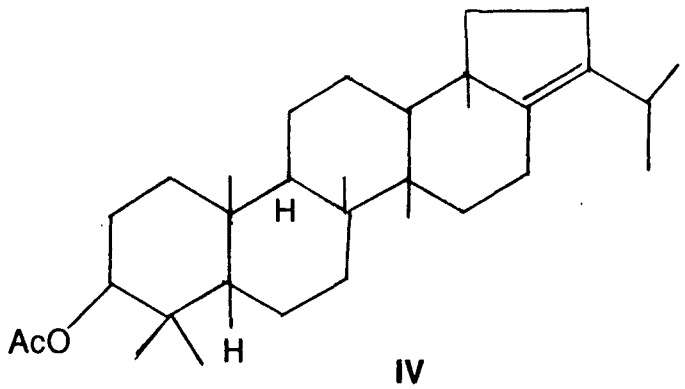
III

RESULTS AND DISCUSSION

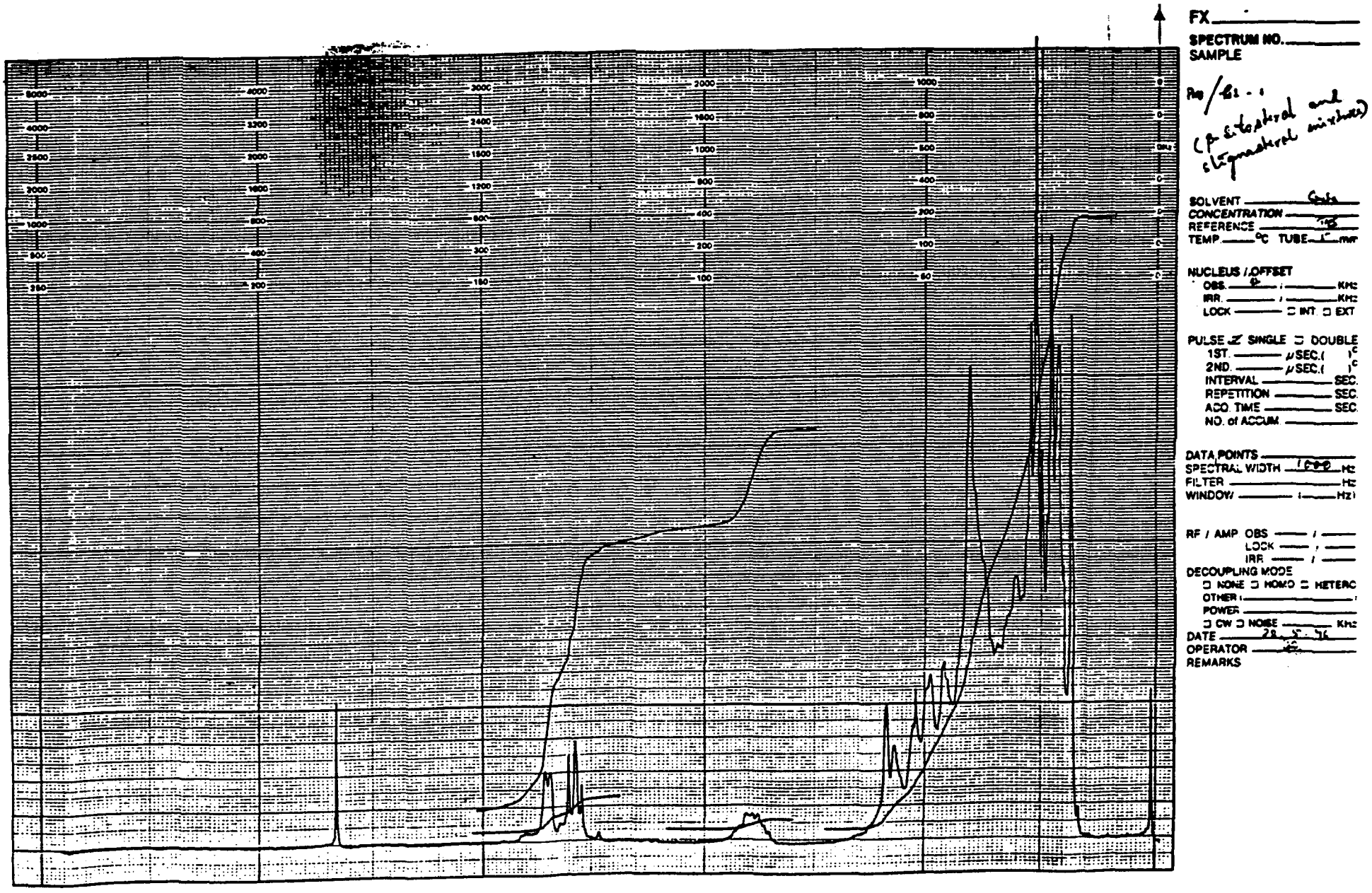
The dried and powdered roots of *Gerbera lanuginosa* Benth. (Asteraceae) were extracted with methanol. The methanol extract was concentrated in vacuum and dissolved in chloroform. The chloroform soluble fraction was subjected to column chromatography on silica gel. Elution of the column with petrol afforded **GL-1.**

GL-1 was obtained as colourless crystals from benzene - chloroform (8:2) and it melted at 157-58°C. It was UV inactive and was detected by iodine and perchloric acid on TLC plate (silica gel, Rf = 0.86, petrol - benzene, 1:1).Its IR spectrum showed a broad band at 3400 - 3250 cm^{-1} indicating the presence of OH group. Other bands were comparable with the IR spectrum of β-sitosterol but from the ^1H NMR spectrum (100 MHz, $CDCl_3$, Fig.1) it was found to be a mixture of **β-sitosterol** and **stigmasterol**. The occurrence of both compounds have not been reported from this plant.

Further elution of column with petrol yielded **GL-2**, crystallized from benzene as a colourless compound, melting at 239°C. Its homogeniety was checked by thin layer chromatography (silica gel, Rf=0.26, petrol - benzene, 1:1) and it was detected by iodine and perchloric acid. The IR spectral data and ^1H NMR spectrum of **GL-2** (100 MHz, $CDCl_3$, Fig.2) showed it to be **hopane** type



IV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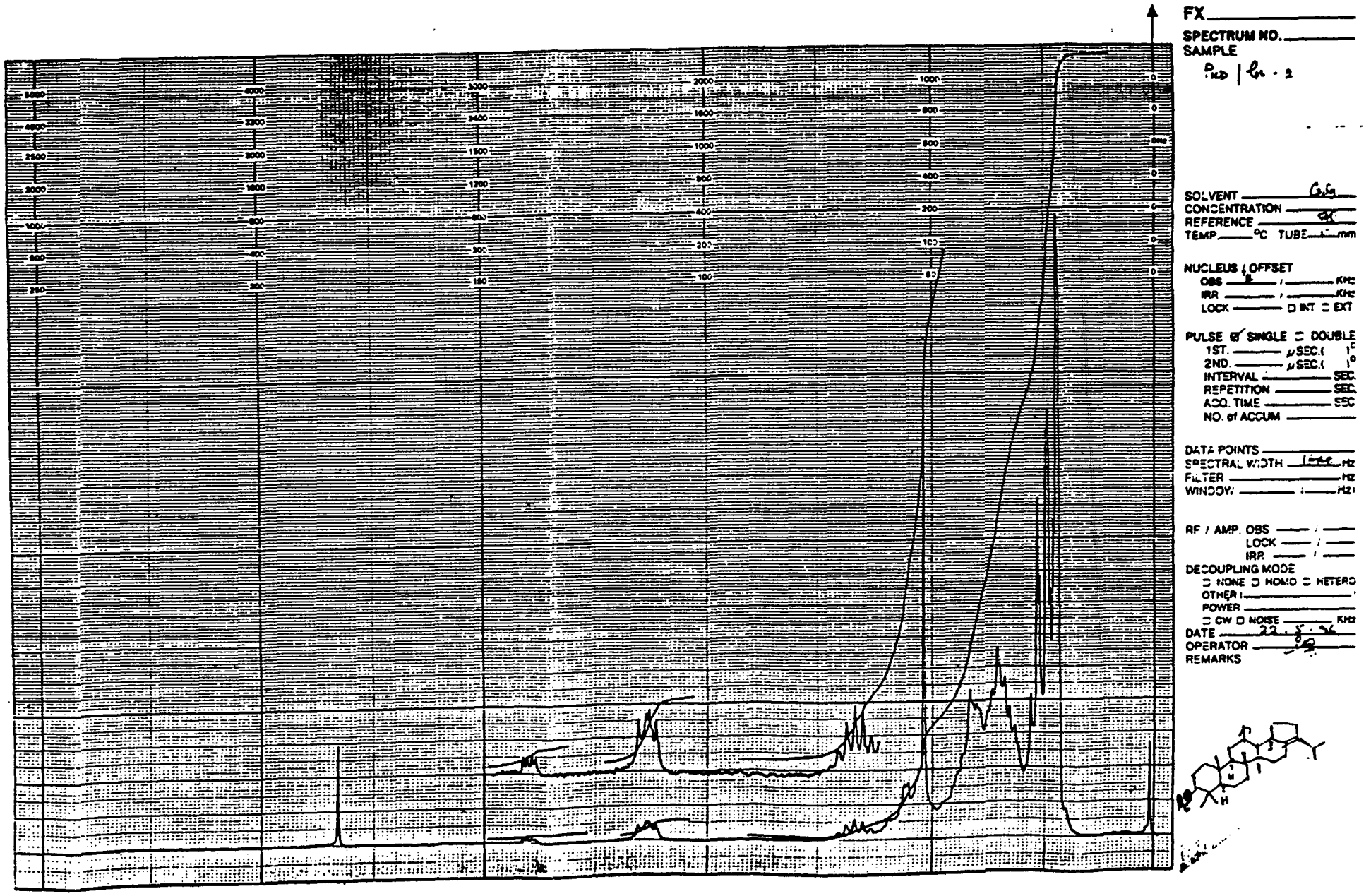
SAMPLE
SOLVENT
CONCENTRATION
REFERENCE
TEMP. °C TUBE
NUCLEUS / OFFSET
OBS. / KHz
IRR. / KHz
LOCK ☐ INT ☐ EXT
PULSE ☑ SINGLE ☐ DOUBLE
1ST. μSEC.( )°
2ND. μSEC.( )°
INTERVAL SEC.
REPETITION SEC.
ACQ. TIME SEC.
NO. of ACCUM
DATA POINTS
SPECTRAL WIDTH Hz
FILTER Hz
WINDOW Hz
RF / AMP OBS /
LOCK /
IRR /
DECOUPLING MODE
☐ NONE ☐ HOMO ☐ HETERO
OTHER
POWER
☐ CW ☐ NOISE KHz
DATE
OPERATOR
REMARKS

Fig.1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SAMPLE
SOLVENT
CONCENTRATION
REFERENCE
TEMP °C TUBE mm
NUCLEUS / OFFSET
OBS / KHz
IRR / KHz
LOCK ☐ INT ☐ EXT
PULSE ☑ SINGLE ☐ DOUBLE
1ST. μSEC. 1°
2ND. μSEC. 1°
INTERVAL SEC.
REPETITION SEC.
ACQ. TIME SEC
NO. of ACCUM
DATA POINTS
SPECTRAL WIDTH Hz
FILTER Hz
WINDOW Hz
RF / AMP. OBS
LOCK
IRR
DECOUPLING MODE
☐ NONE ☐ HOMO ☐ HETERO
OTHER
POWER
☐ CW ☐ NOISE KHz
DATE
OPERATOR
REMARKS

Fig.2

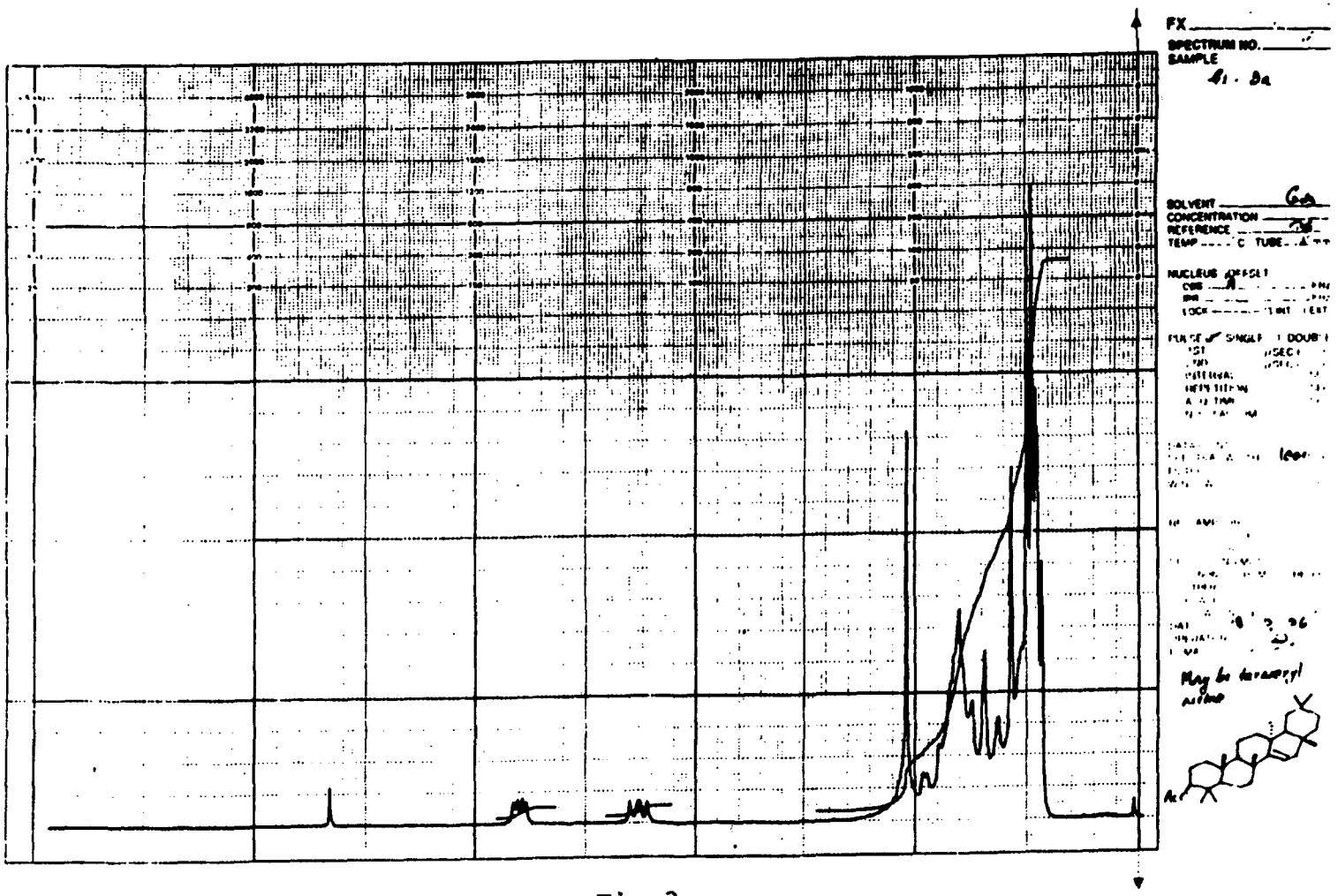
FX
SPECTRUM NO.
SAMPLE
SOLVENT
CONCENTRATION
REFERENCE
TEMP
TUBE
NUCLEUS
PULSE
SINGLE
DOUBLE

Fig.3

triterpene as shown in structure **IV**. However, the ^1H NMR also shows some additional peaks, probably arising from the contamination of taraxeryl acetate. Although hopane type triterpenes have not yet been reported from this plant, the structural determination is not conclusive and efforts to isolate pure sample of **GL-2** is in progress.

Further elution of column with petrol - benzene (1:1) yielded **GL-3** as colourless compound from benzene. Its melting point was 314°C and it showed Rf value of 0.84 on TLC·(silica gel, petrol - benzene, 1:1) and was detected by iodine and perchloric acid. In the infra red spectrum a band appeared at 3450 cm^{-1} indicating the presence of OH group, while a band appearing at 1720 cm^{-1} may be due to C=O stretching vibration of carboxylic or ester functional group. The ^1H NMR spectrum (100 MHz, $CDCl_3$, Fig.3) of **GL-3** acetate is indicative of a pentacyclic triterpene skeleton similar to taraxeryl acetate but the melting point of the parent compound **GL-3** (314°C), melting point of taraxerol (282-84°C) and the appearance of band at 1720 cm^{-1} in IR differ from **taraxerol**. Therefore, the structure (**Ia**) assigned to **GL-3** is not conclusive.

Further elution of the column with petrol - benzene (1:1) yielded a colourless compound, **GL-4**, crystallized from benzene, melting at 89°C. Its IR spectrum showed C-H stretching bands at 2900 and 2800, (-CH_2-, -CH_3), 2300 (C-O stretching and O-H deformation) and 1700 cm^{-1} (C=O stretching) indicating it to be a fatty acid. It analysed for $C_{30}H_{60}O_2$ (M^+ 452). In the MS (Fig.4) molecular ion appeared at 452 (4) and base peak at m/z 57. Other prominent peaks due to successive fragmentation of the chain appeared at m/z 428 (48), 396 (10), 368 (20), 129 (30), 83 (30) and 74 (66).The ^1H NMR spectrum (600 MHz, $CDCl_3$, Fig. 5) showed a triplet at δ 0.867 (J=7 Hz) integrating for three

GL4
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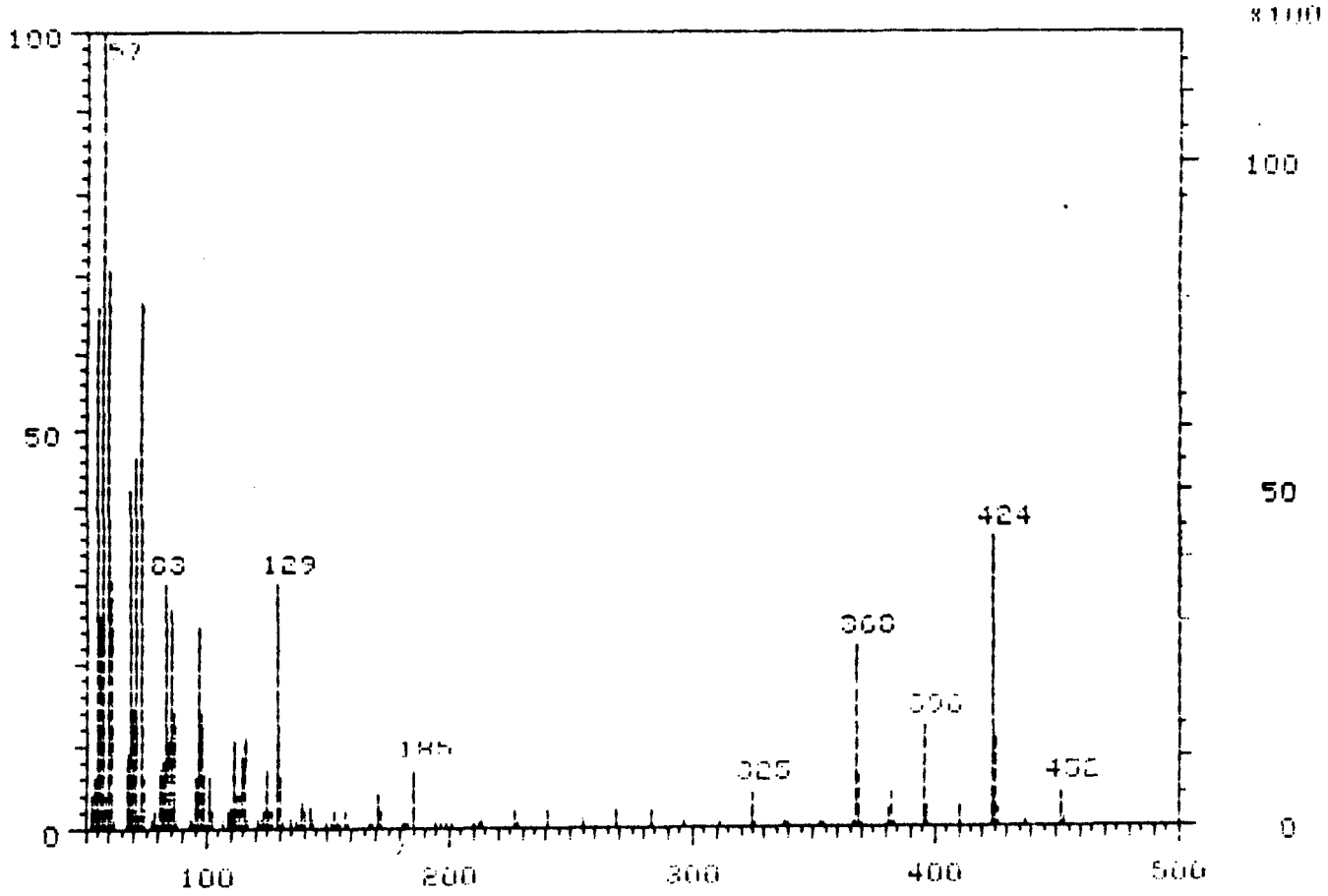
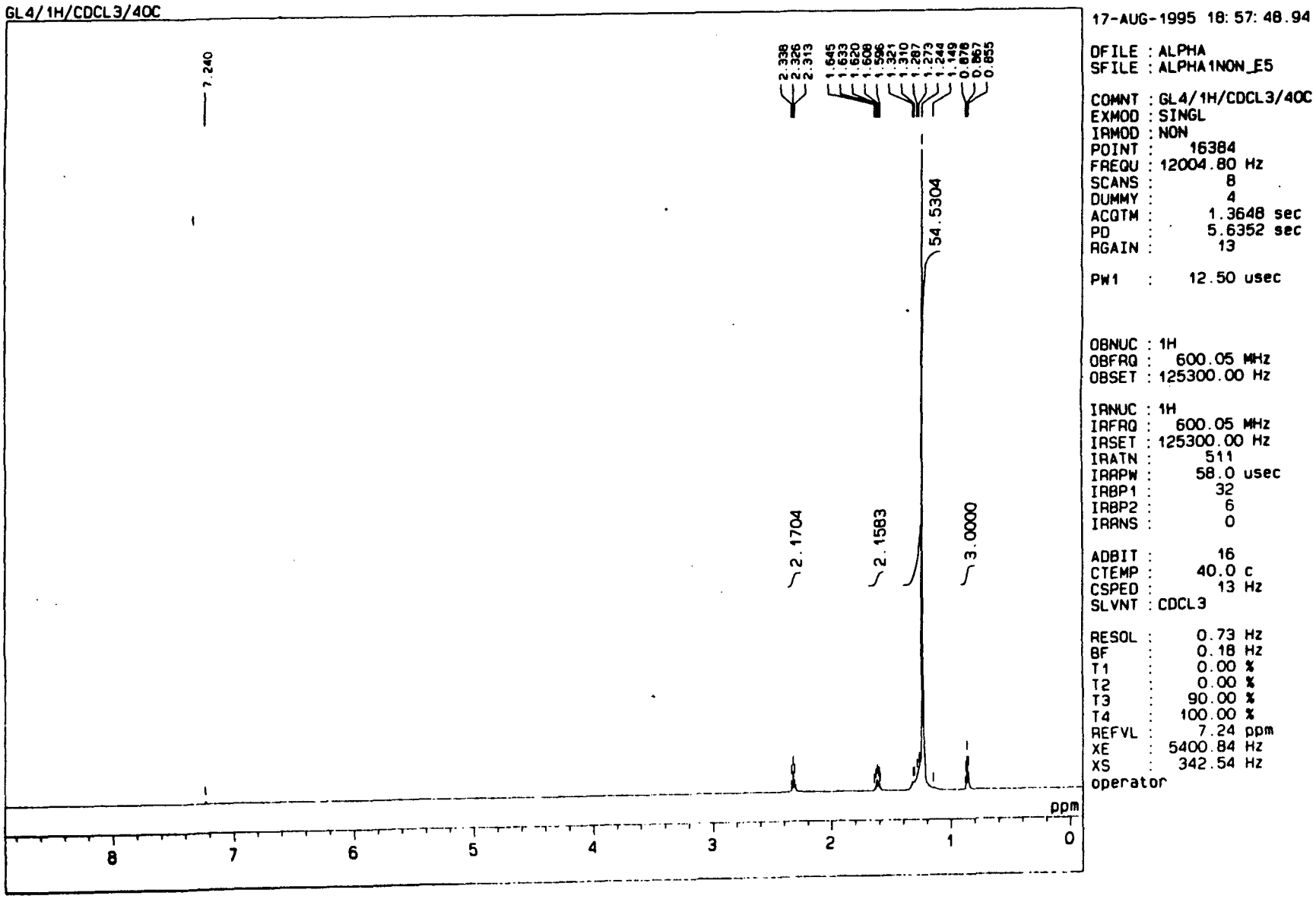


Fig.4


GL4/1H/CDCL3/40C
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2.338
2.326
2.313
1.645
1.633
1.620
1.608
1.596
1.321
1.310
1.287
1.273
1.244
1.149
0.878
0.867
0.855
54.5304
2.1704
2.1583
3.0000
ppm
8
7
6
5
4
3
2
1
0
17-AUG-1995 18:57:48.94
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IRMOD : NON
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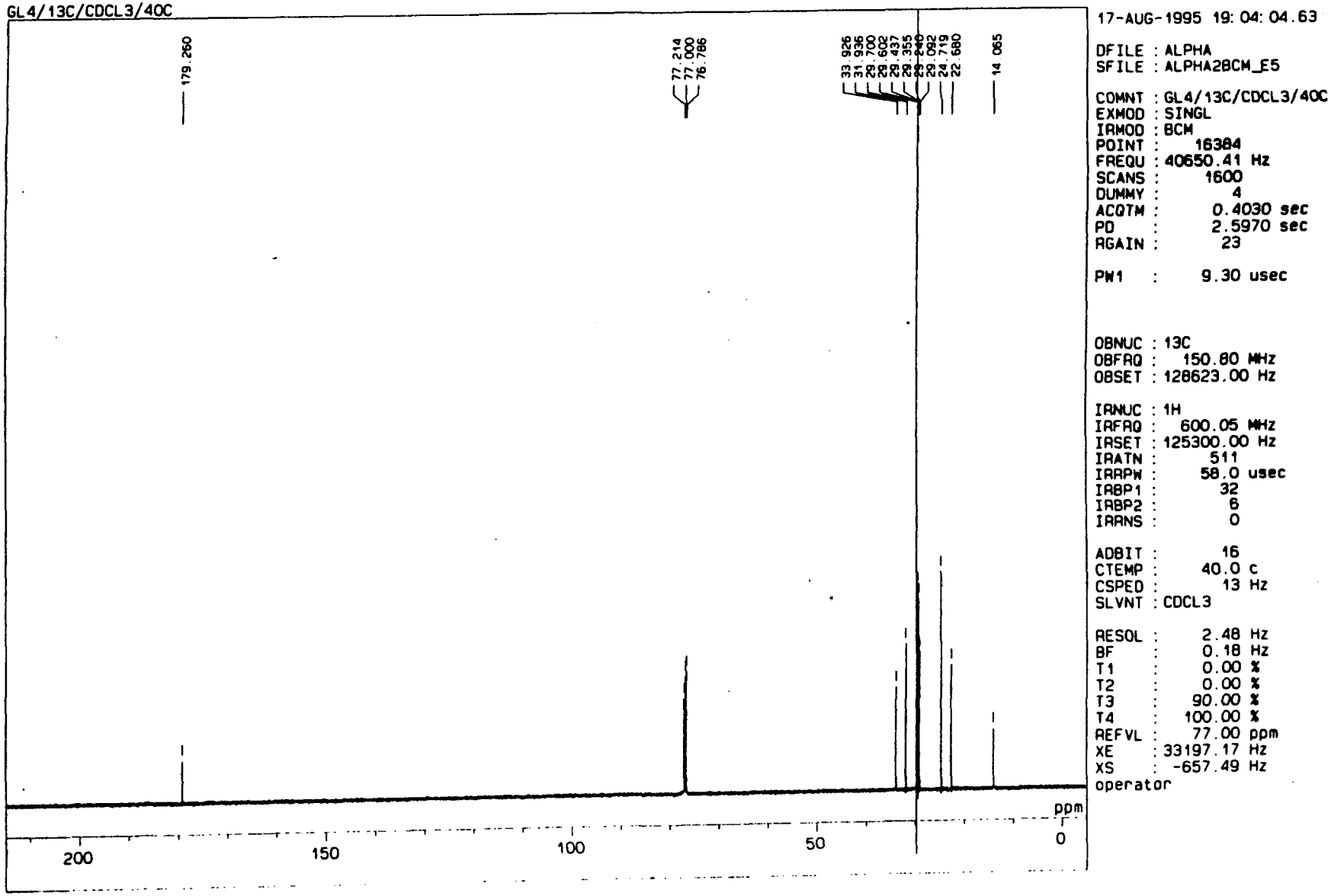
ACQTM : 1.3648 sec
PD : 5.6352 sec
RGAIN : 13
PW1 : 12.50 usec
OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125300.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRANS : 0
ADBIT : 16
CTEMP : 40.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 0.73 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 7.24 ppm
XE : 5400.84 Hz
XS : 342.54 Hz
operator

Fig.5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77.214
77.000
76.786
33.926
31.936
29.700
29.602
29.437
29.355
29.240
29.092
24.719
22.680
14.065
ppm
200
150
100
50
0
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IRMOD : BCM
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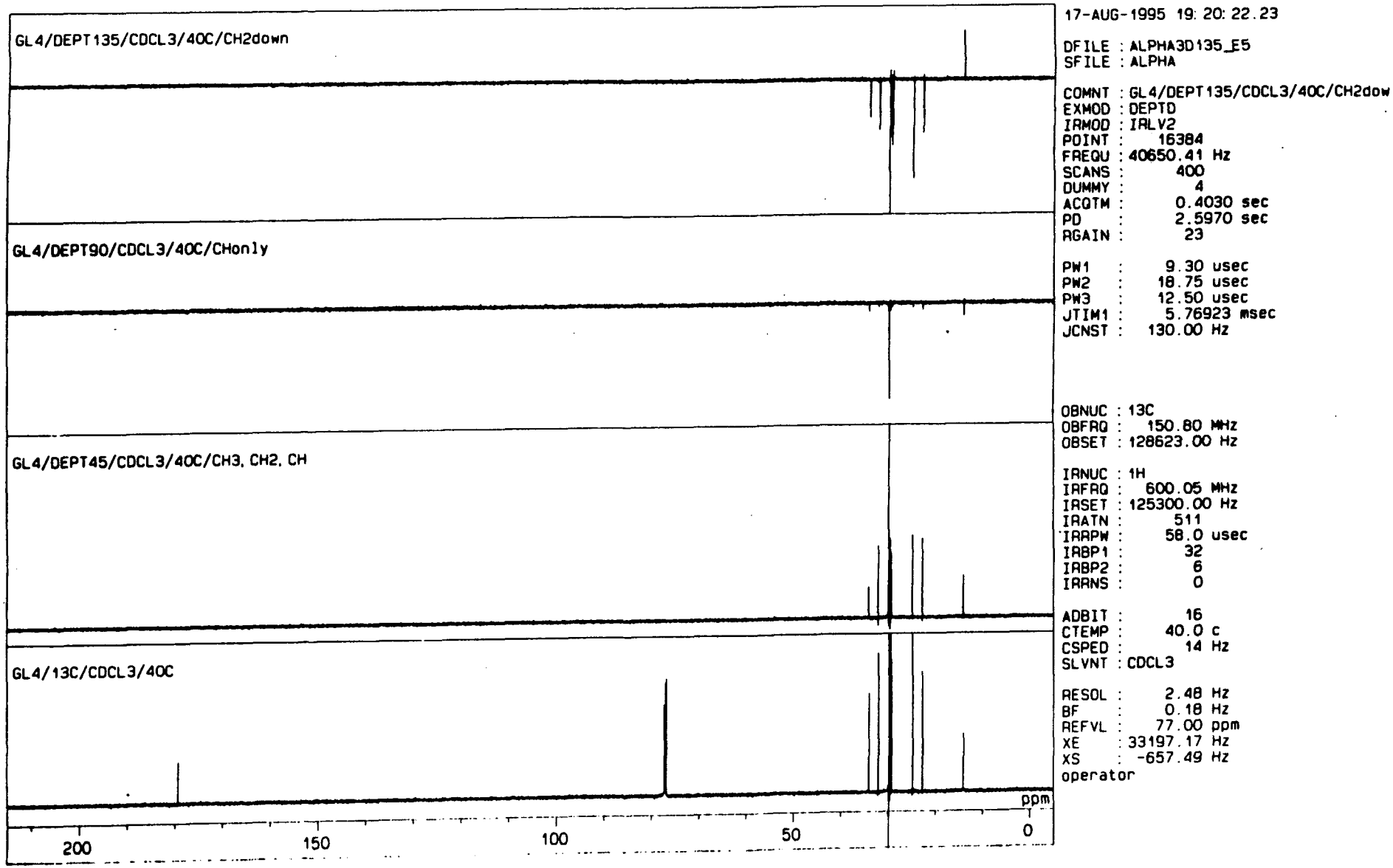
SCANS : 1600
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23
PW1 : 9.30 usec
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
ADBIT : 16
CTEMP : 40.0 c
CSPED : 13 Hz
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
T3 : 90.00 %
T4 : 100.00 %
REFVL : 77.00 ppm
XE : 33197.17 Hz
XS : -657.49 Hz
operator

Fig.6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GL4/DEPT45/CDCL3/40C/CH3, CH2, CH
GL4/13C/CDCL3/40C
ppm
200
150
100
50
0
17-AUG-1995 19:20:22.23
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IRMOD : IRLV2
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 400
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23
PW1 : 9.30 usec
PW2 : 18.75 usec
PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0
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CTEMP : 40.0 c
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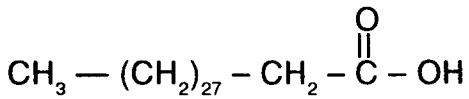
SLVNT : CDCL3
RESOL : 2.48 Hz
BF : 0.18 Hz
REFVL : 77.00 ppm
XE : 33197.17 Hz
XS : -657.49 Hz
operator

Fig.7

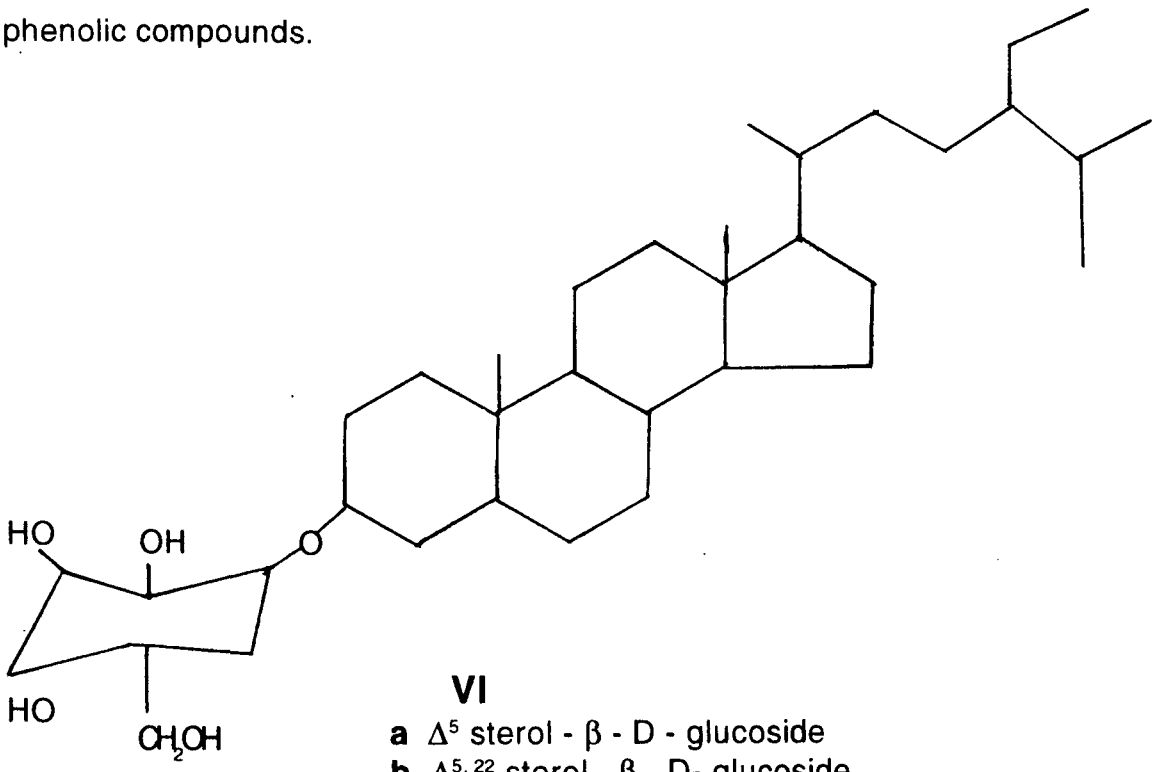
methyl protons, multiplet at δ 1.149-1.321 integrating for 55 protons of methylene envelope, multiplet at δ 1.596 - 1.645 integrating for two methylene protons at C-3 and triplet at δ 2.326 integrating for two protons of C-2. The ^{13}C NMR spectrum (150.8 MHz, $CDCl_3$, Fig. 6) showed signals at δ 14.065 for terminal methyl carbon, δ 33.926 for C-2 and δ 179.260 for carboxylic carbon. The other methylene carbons showed signals at δ 22.680 (C-29), 24.719 (C-3), 29.092-29.700 (C-4 to C-27) and 31.936 (C-28).

The assignments were confirmed by DEPT experiments (150.8 MHz, $CDCl_3$, Fig. 7). On the basis of above spectral data **GL-4** was characterized as long chain fatty acid **triacontanoic acid (v)**



V

Further elution of column with benzene yielded inseparable mixture of phenolic compounds.



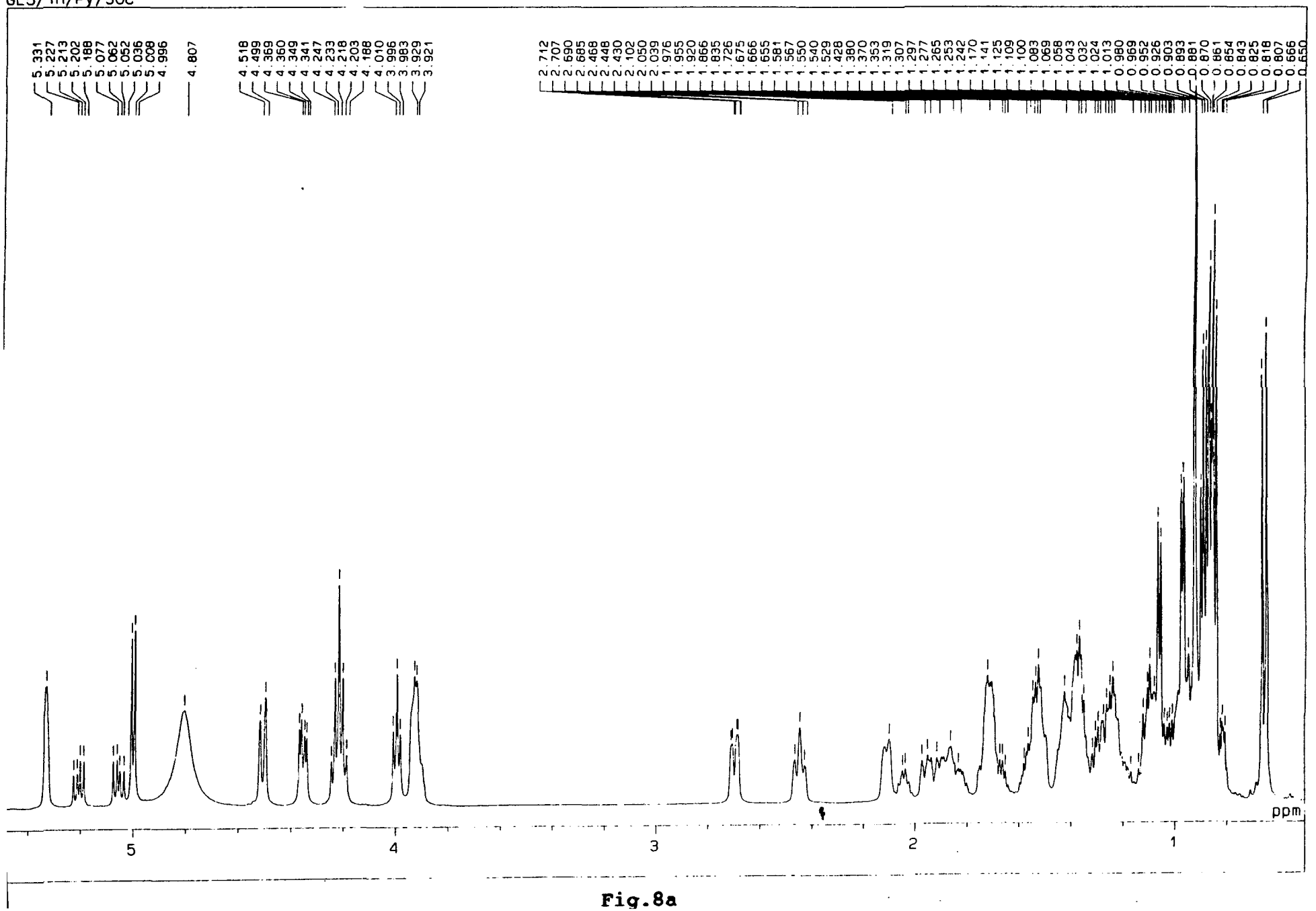
VI

a Δ^5 sterol - β - D - glucoside

b $\Delta^{5,22}$ sterol - β - D- glucoside

Table I. ^{13}C NMR data of compound GL-5 (150.8 MHz, C_5D_5N)

Carbon	Chemical Shift (δ,ppm)	
	Δ^{13} sterol-β-D-glucoside	$\Delta^{5,22}$ sterol-β-D-glucoside
1	37.516	36.957
2	32.092	32.173
3	71.729	71.729
4	42.382	42.514
5	140.976	138.806
6	121.905	129.517
7	32.090	30.265
8	32.173	32.090
9	50.389	51.441
10	36.398	36.957
11	21.486	21.289
12	39.867	39.374
13	42.382	42.514
14	56.866	56.965
15	24.528	24.561
16	28.556	28.556
17	56.126	56.291
18	12.000	12.000
19	19.448	19.217
20	36.398	36.398
21	19.037	19.267
22	34.244	32.173
23	29.295	29.526
24	46.081	46.081
25	26.468	25.695
26	19.037	19.037
27	19.990	19.990
28	23.443	23.443
29	12.000	12.181
1'	102.571	102.571
2'	75.313	71.729
3'	78.420	78.583
4'	71.729	71.729
5'	78.157	78.420
6'	62.851	62.851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5.227
5.213
5.202
5.188
5.077
5.062
5.052
5.036
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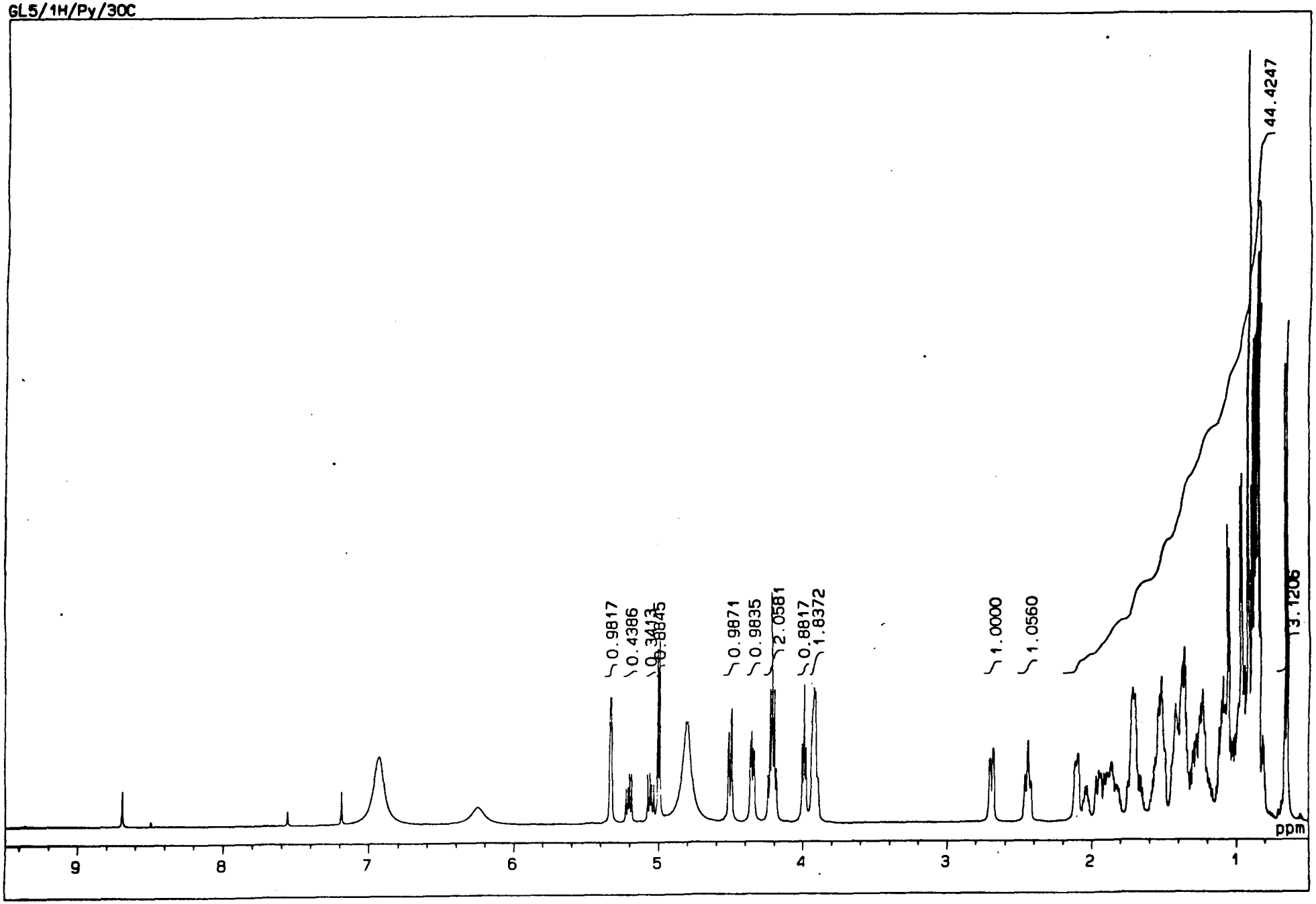
4.996
4.807
4.518
4.499
4.369
4.360
4.349
4.341
4.247
4.233
4.218
4.203
4.188
4.010
3.996
3.983
3.929
3.921
2.712
2.707
2.690
2.685
2.468
2.448
2.430
2.102
2.050
2.039
1.976
1.955
1.920
1.866
1.835
1.726
1.675
1.666
1.655
1.581
1.567
1.550
1.540
1.529
1.428
1.380
1.370
1.353
1.319
1.307
1.297
1.277
1.265
1.253
1.242
1.170
1.141
1.125
1.109
1.100
1.083
1.069
1.058
1.043
1.032
1.024
1.013
0.980
0.969
0.952
0.926
0.903
0.893
0.881
0.870
0.861
0.854
0.843
0.825
0.818
0.807
0.666
0.650
ppm
5
4
3
2
1

Fig.8a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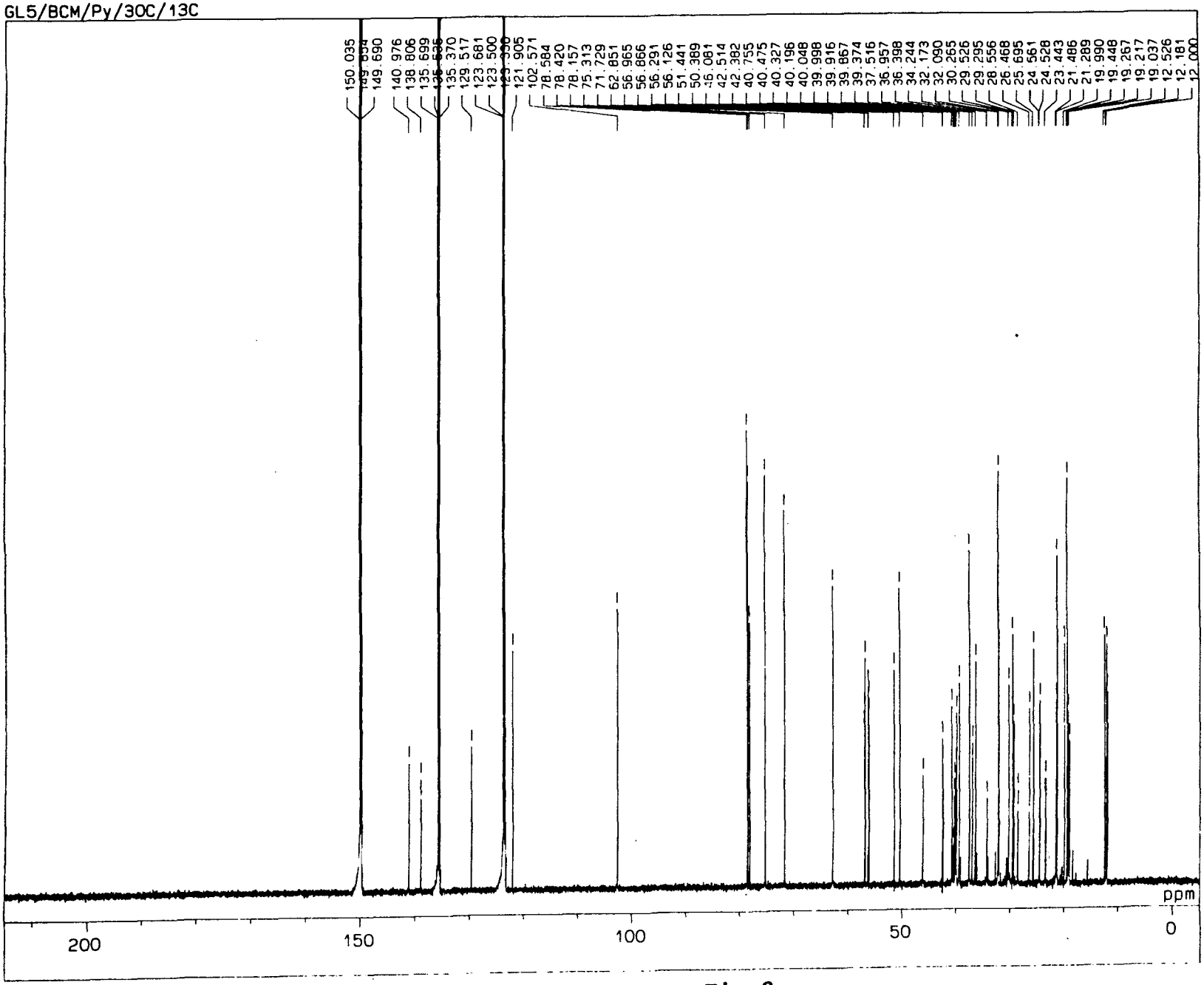
0.9835
2.0581
0.8817
1.8372
1.0000
1.0560
44.4247
13.1206
ppm
9
8
7
6
5
4
3
2
1

Fig.8b



18-AUG-1995 10: 43: 27.24

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PD : 2.5970 sec
RGAIN : 23

PW1 : 9.30 usec

OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 29.9 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 2.48 Hz
BF : 0.18 Hz
T1 : 0.00 %
T2 : 0.00 %
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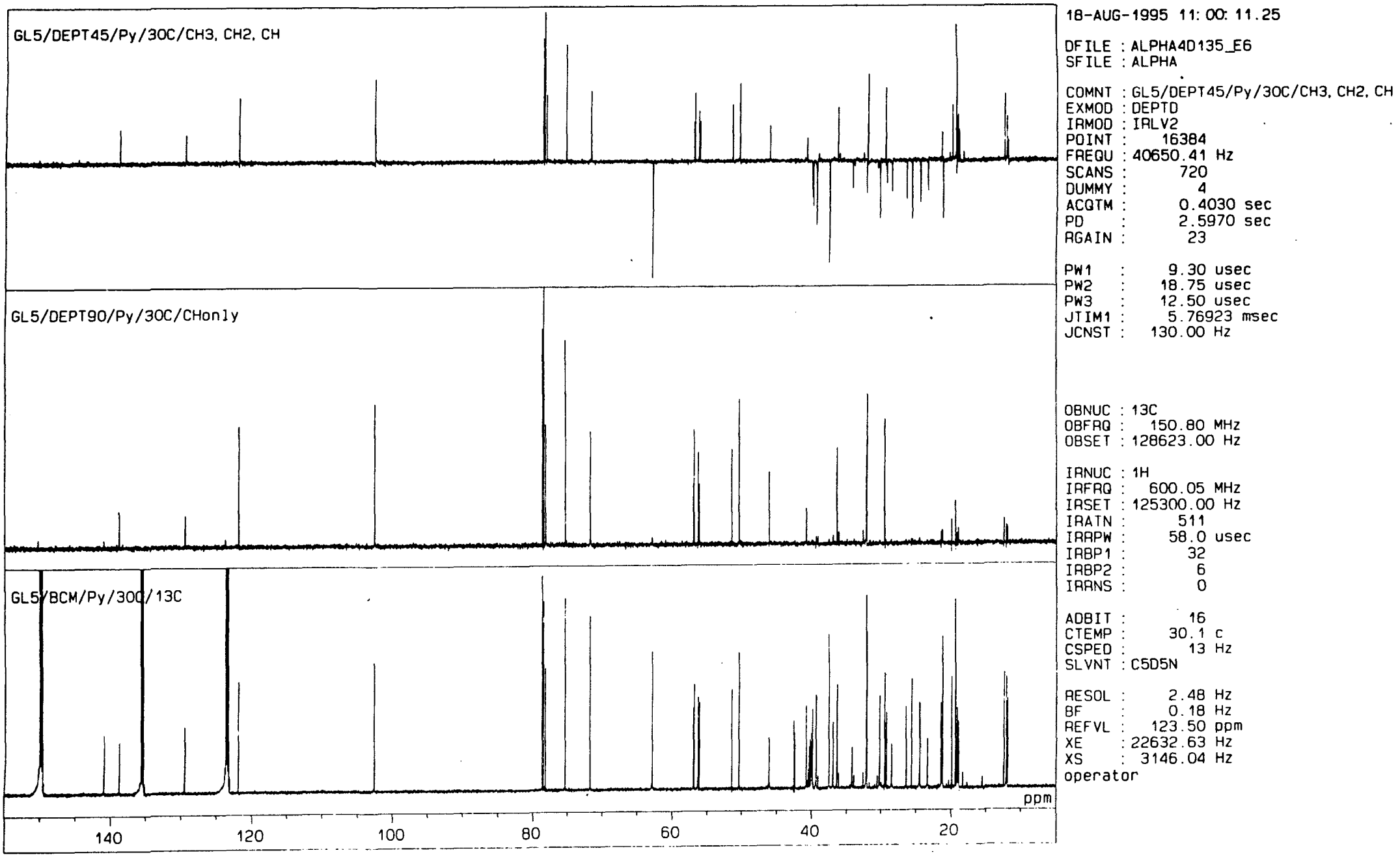
T4 : 100.00 %
REFVL : 123.50 ppm
XE : 33199.65 Hz
XS : -626.48 Hz
operator

Fig.9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GL5/BCM/Py/30C/13C
ppm
140
120
100
80
60
40
20
18-AUG-1995 11:00:11.25
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PD : 2.5970 sec
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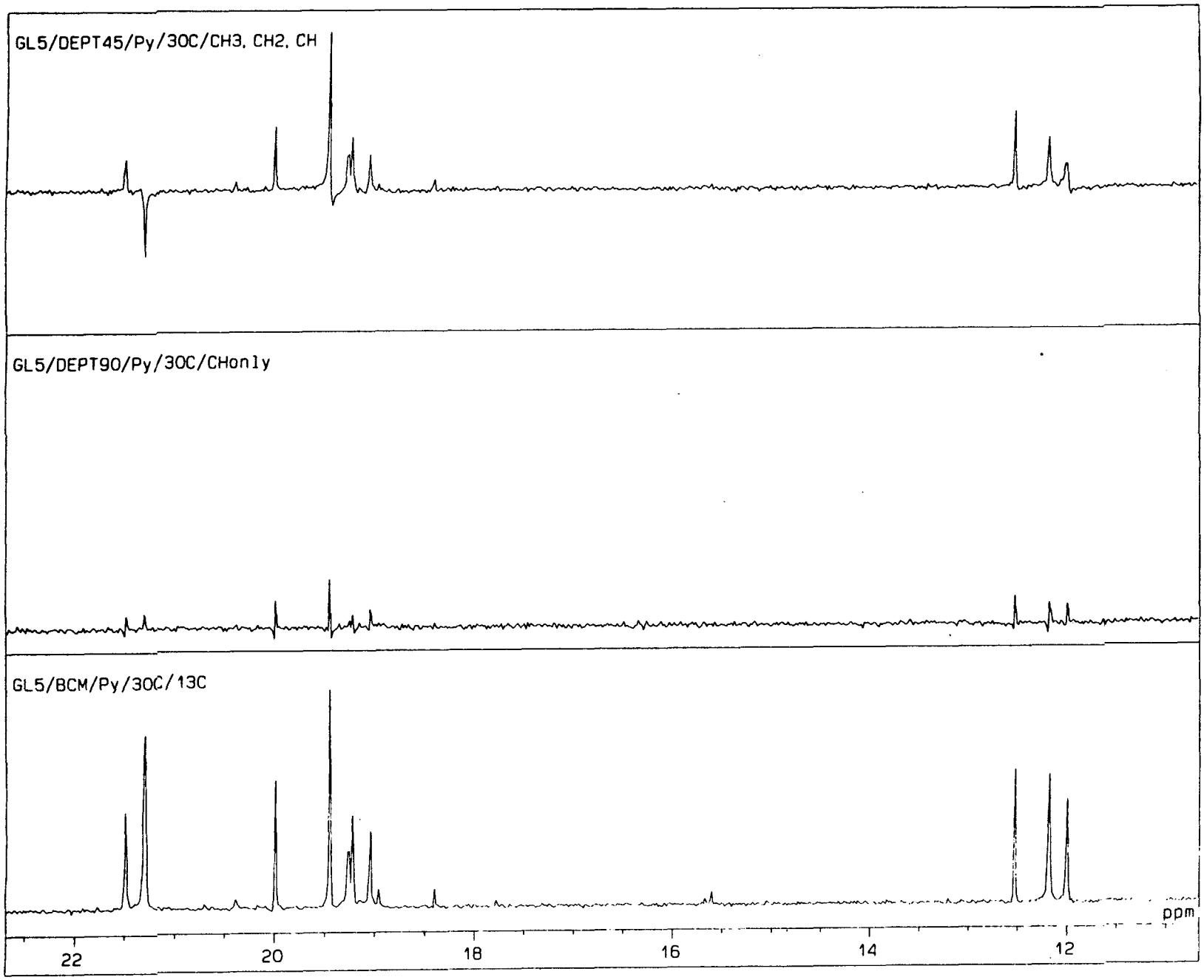
PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz
IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRAPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRANS : 0
ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
SLVNT : C5D5N
RESOL : 2.48 Hz
BF : 0.18 Hz
REFVL : 123.50 ppm
XE : 22632.63 Hz
XS : 3146.04 Hz
operator

Fig.10a



18-AUG-1995 11:01:22.46

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RGAIN : 23

PW1 : 9.30 usec
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PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz

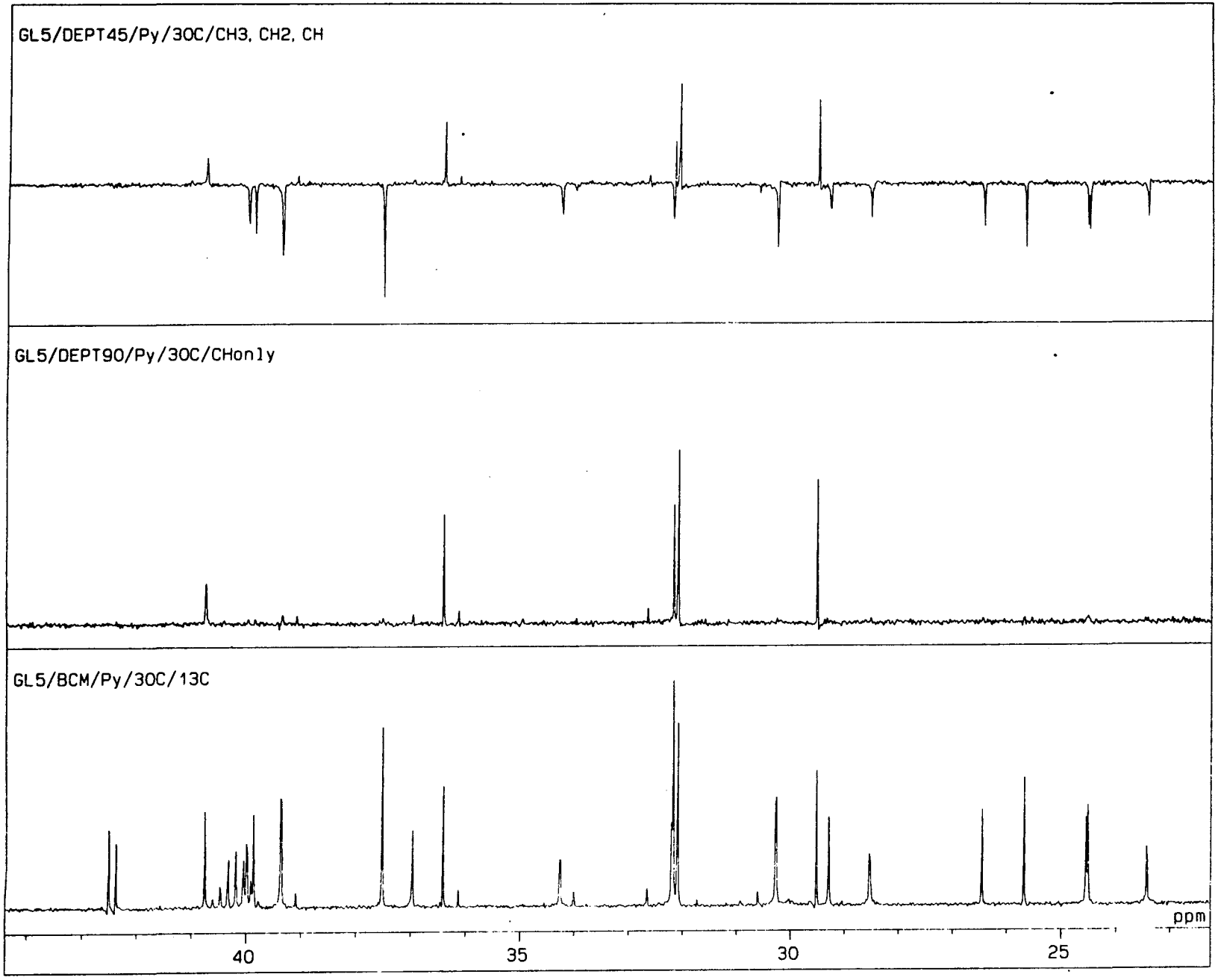
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 2.48 Hz
BF : 0.18 Hz
REFVL : 123.50 ppm
XE : 1813.69 Hz
XS : 12699.53 Hz
operator

Fig.10b



18-AUG-1995 11: 01: 55.43

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FREQU : 40650.41 Hz
SCANS : 720
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23

PW1 : 9.30 usec
PW2 : 18.75 usec
PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz

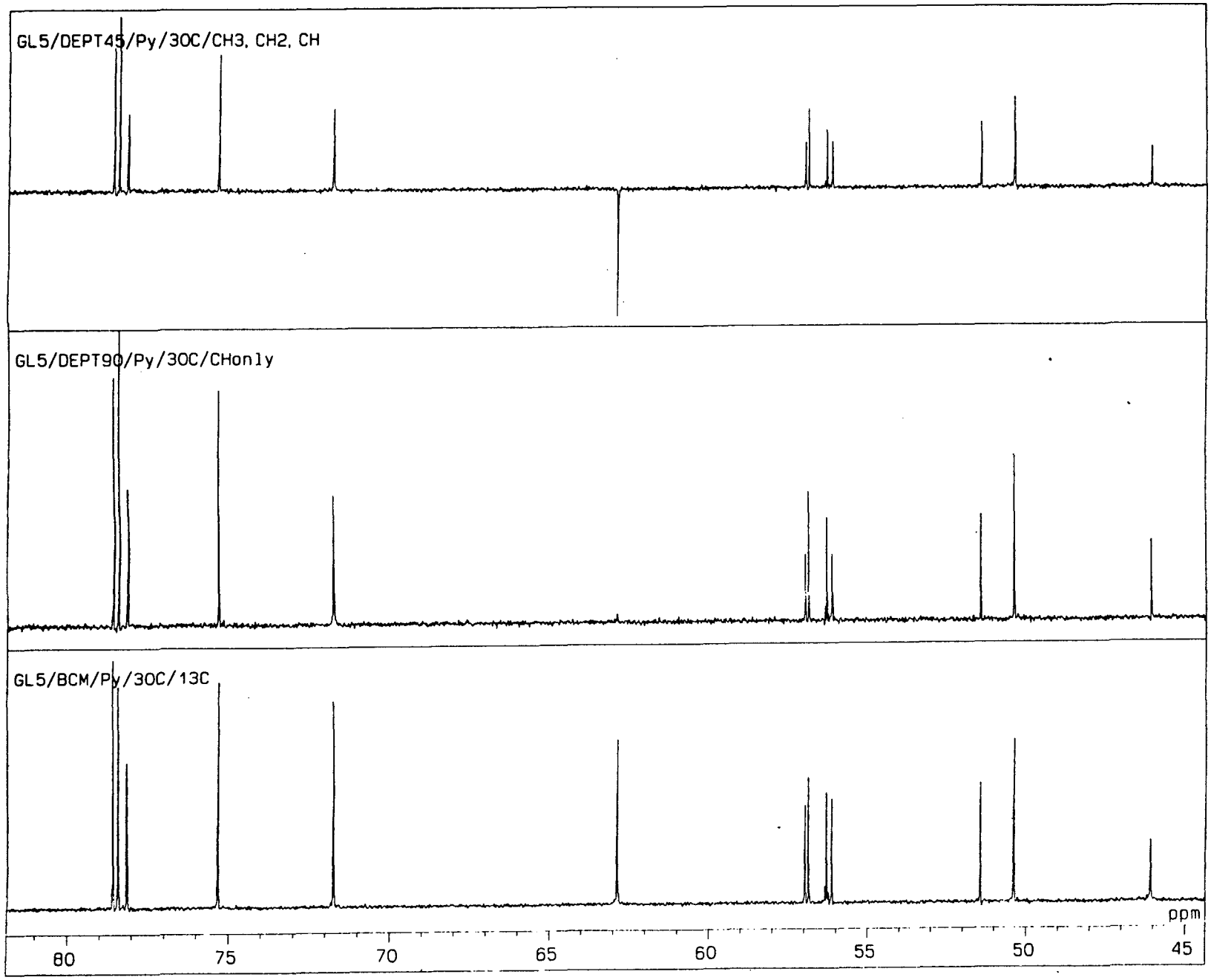
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 2.48 Hz
BF : 0.18 Hz
REFVL : 123.50 ppm
XE : 3339.57 Hz
XS : 10187.41 Hz
operator

Fig.10c



18-AUG-1995 11: 02: 18.35

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IRMOD : IRLV2
POINT : 16384
FREQU : 40650.41 Hz
SCANS : 720
DUMMY : 4
ACQTM : 0.4030 sec
PD : 2.5970 sec
RGAIN : 23

PW1 : 9.30 usec
PW2 : 18.75 usec
PW3 : 12.50 usec
JTIM1 : 5.76923 msec
JCNST : 130.00 Hz

OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 128623.00 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 2.48 Hz
BF : 0.18 Hz
REFVL : 123.50 ppm
XE : 5659.40 Hz
XS : 5690.41 Hz
operator

Fig.10d

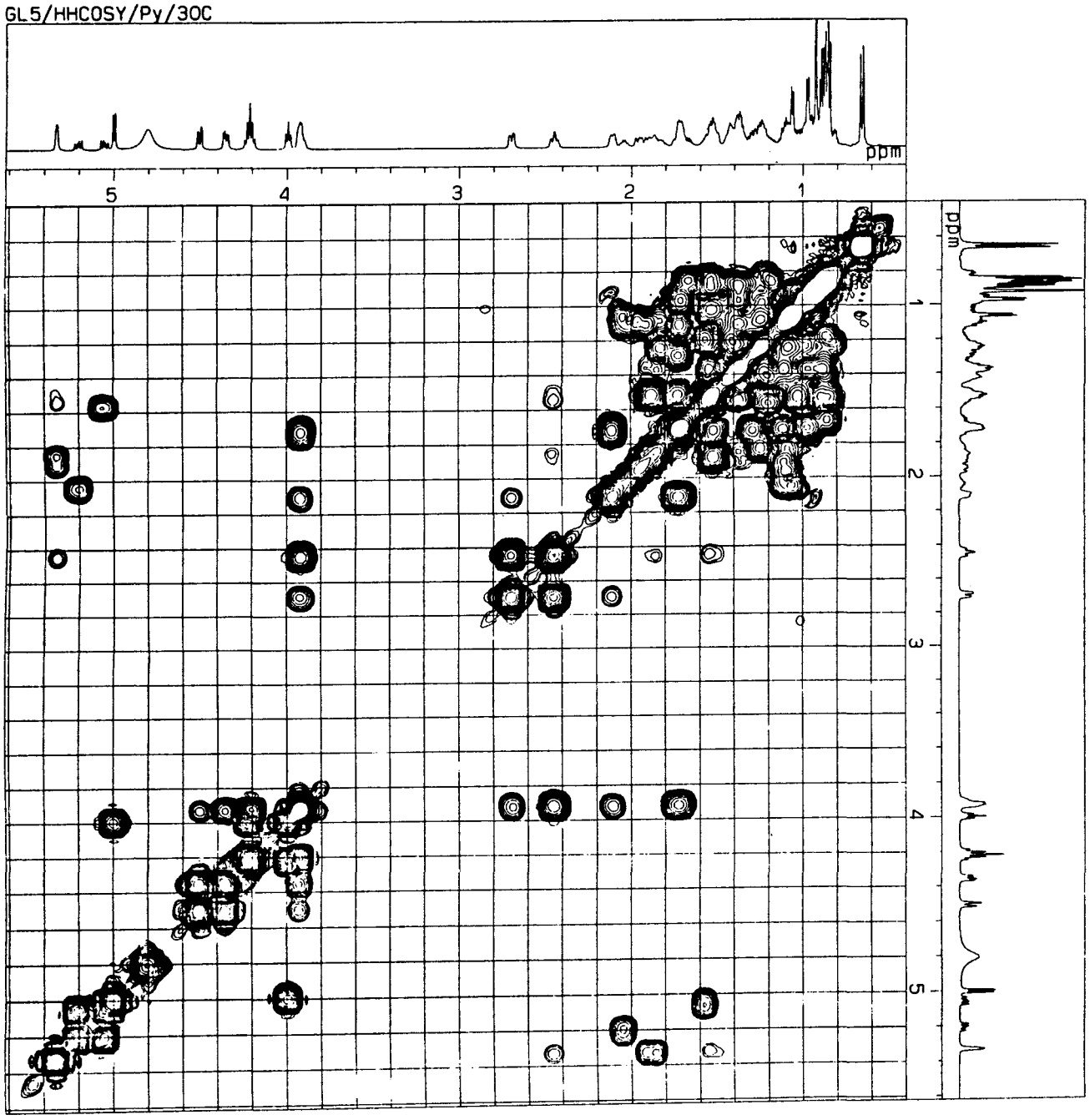


Fig.11

18-AUG-1995 10: 25: 15.64

DFILE : ALPHA
SFILE : ALPHA2COSY_E6
HR2FILE: ALPHA1NON_E6
HR1FILE: ALPHA1NON_E6

COMNT : GL5/HHCOSY/Py/30C
EXMOD : COSY
IRMOD : NON
POINT : 512
FREQU : 5924.17 Hz
SCANS : 8
DUMMY : 4
ACQTM : 0.0432 sec
PD : 0.9568 sec
RGAIN : 10

CLFRQ : 5924.17 Hz
CLPNT : 512
TOSCN : 256
CINWT : 10.00 usec
CINTV : 168.80 usec

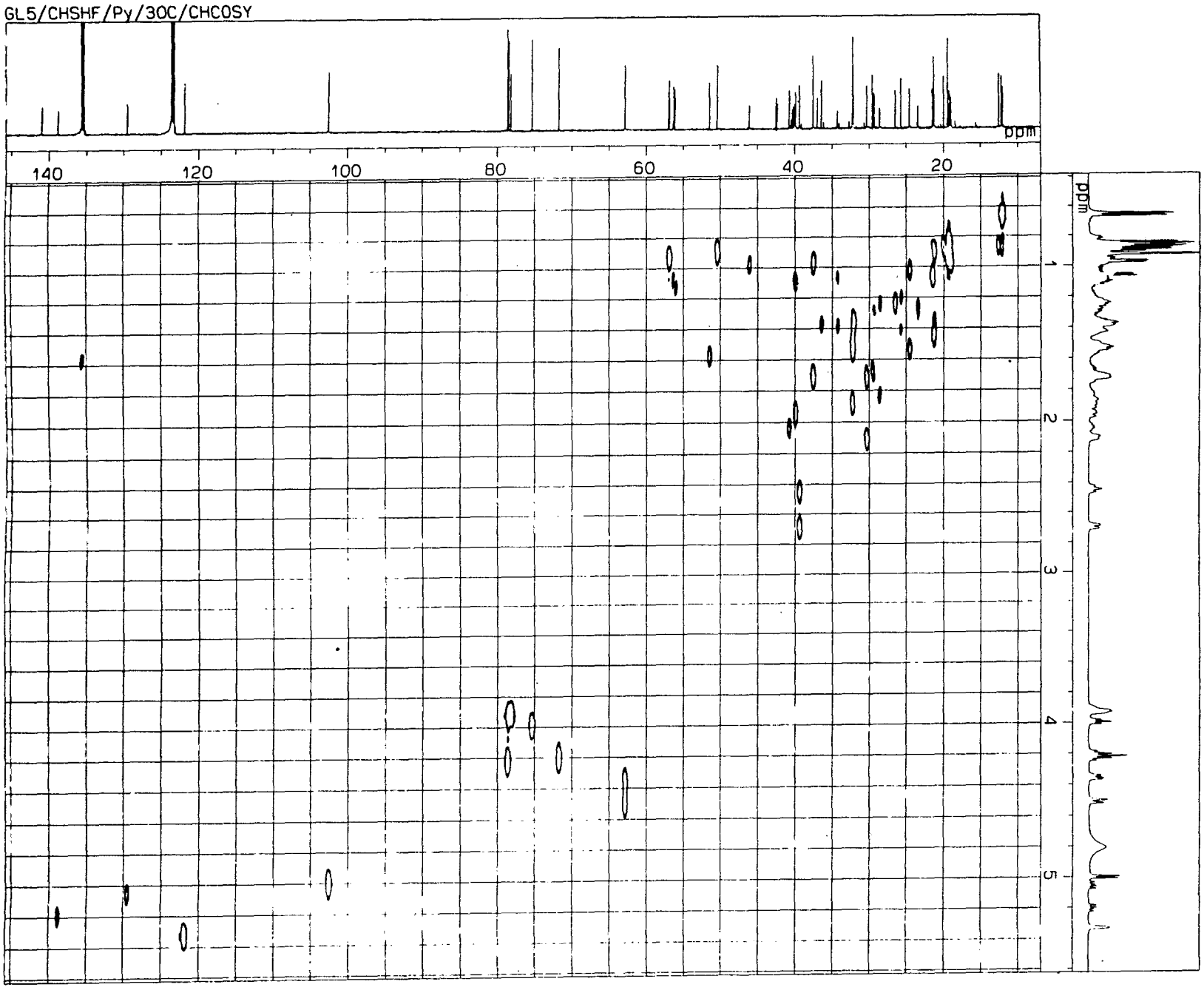
PW1 : 12.50 usec
PW2 : 20.00 usec
PI1 : 120.0000 msec
PI2 : 1.0000 msec

OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125037.69 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125300.00 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 29.9 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 11.57 Hz
CLRSO : 11.57 Hz
TLINE : 16
THTOP : 6.0000
THBTM : 0.0400
operator



18-AUG-1995 11: 10: 45.95

DFILE : ALPHA
SFILE : ALPHA5CHSHF_E6
HR2FILE: ALPHA3BCM_E6
HR1FILE: ALPHA1NON_E6

COMNT : GL5/CHSHF/Py/30C/CHCOSY
EXMOD : CHSHF
IRMOD : IRLV2
POINT : 1024
FREQU : 25000.00 Hz
SCANS : 72
DUMMY : 4
ACQTM : 0.0205 sec
PD : 1.4795 sec
RGAIN : 20

CLFRQ : 5921.36 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 84.44 usec

PW1 : 9.30 usec
PW3 : 12.50 usec
PI1 : 120.0000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz

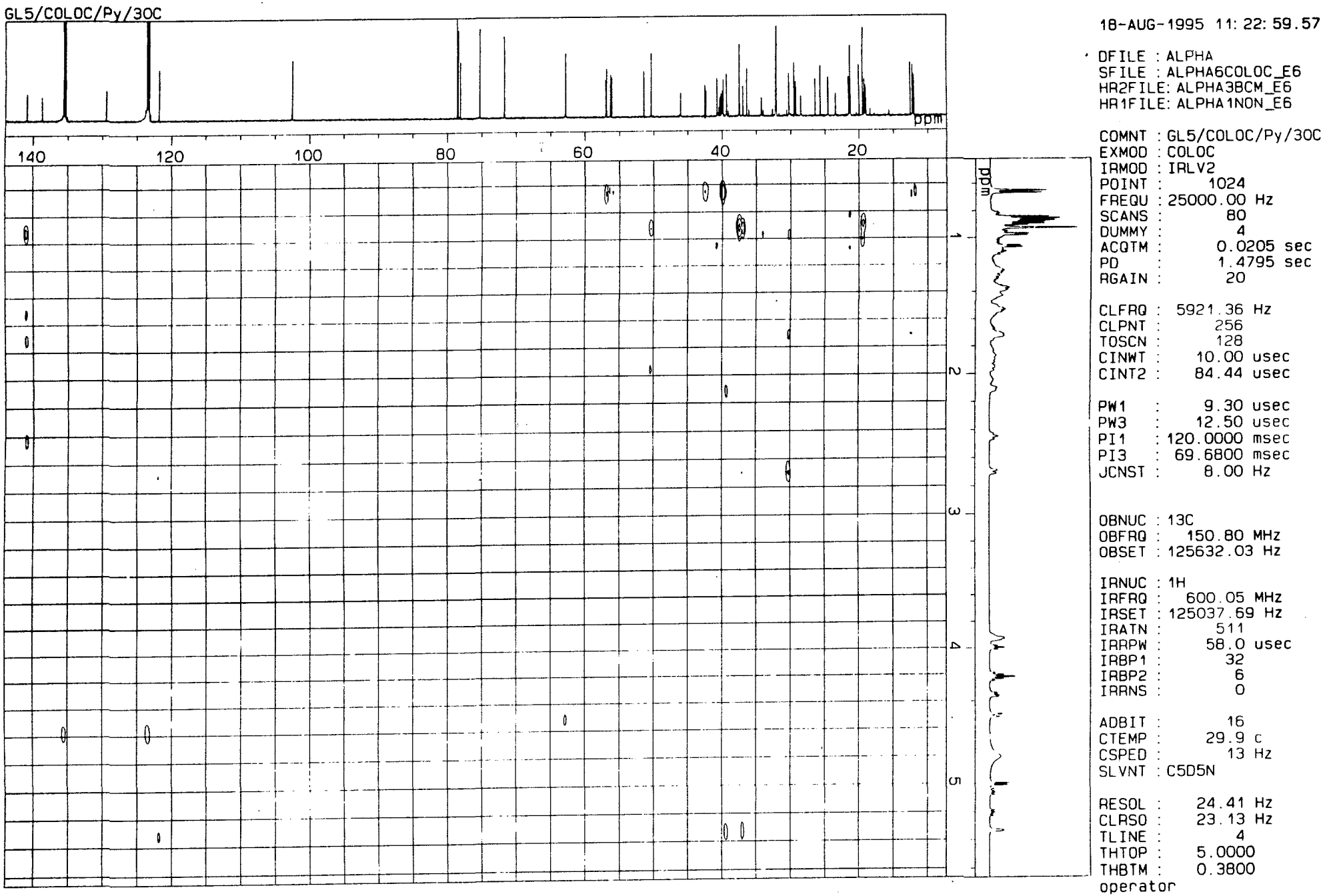
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 125632.03 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125037.69 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 30.1 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 24.41 Hz
CLRSO : 23.13 Hz
TLINE : 12
THTOP : 1.7252
THBTM : 0.9000
operator

Fig.12



18-AUG-1995 11: 22: 59.57

DFILE : ALPHA
SFILE : ALPHA6COLOC_E6
HR2FILE: ALPHA3BCM_E6
HR1FILE: ALPHA1NON_E6

COMNT : GL5/COLOC/Py/30C
EXMOD : COLOC
IRMOD : IRLV2
POINT : 1024
FREQU : 25000.00 Hz
SCANS : 80
DUMMY : 4
ACQTM : 0.0205 sec
PD : 1.4795 sec
RGAIN : 20

CLFRQ : 5921.36 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 84.44 usec

PW1 : 9.30 usec
PW3 : 12.50 usec
PI1 : 120.0000 msec
PI3 : 69.6800 msec
JCNST : 8.00 Hz

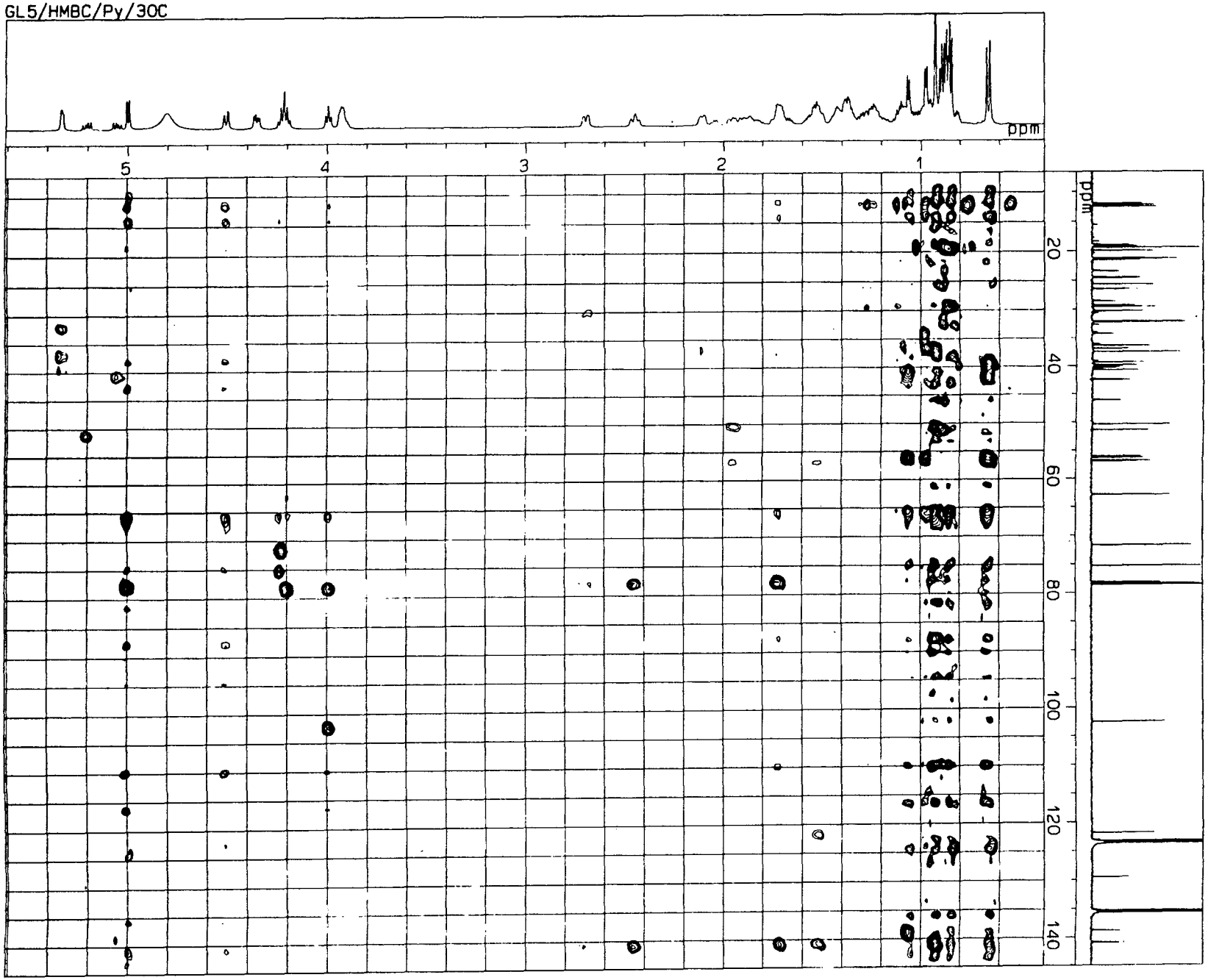
OBNUC : 13C
OBFRQ : 150.80 MHz
OBSET : 125632.03 Hz

IRNUC : 1H
IRFRQ : 600.05 MHz
IRSET : 125037.69 Hz
IRATN : 511
IRRPW : 58.0 usec
IRBP1 : 32
IRBP2 : 6
IRRNS : 0

ADBIT : 16
CTEMP : 29.9 c
CSPED : 13 Hz
SLVNT : C5D5N

RESOL : 24.41 Hz
CLRSO : 23.13 Hz
TLINE : 4
THTOP : 5.0000
THBTM : 0.3800
operator

Fig.13



18-AUG-1995 14: 49: 52.15

DFILE : ALPHA
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HR2FILE: ALPHA1NON_E6
HR1FILE: ALPHA3BCM_E6

COMNT : GL5/HMBC/Py/30C
EXMOD : HMBC
IRMOD : IRLV2
POINT : 512
FREQU : 5871.99 Hz
SCANS : 80
DUMMY : 32
ACQTM : 0.0872 sec
PD : 1.4128 sec
RGAIN : 11

CLFRQ : 25000.00 Hz
CLPNT : 256
TOSCN : 128
CINWT : 10.00 usec
CINT2 : 20.00 usec

PW1 : 12.50 usec
PW3 : 9.30 usec
PI1 : 62.5000 msec
PI3 : 69.6800 msec
JCNST : 140.00 Hz

OBNUC : 1H
OBFRQ : 600.05 MHz
OBSET : 125058.94 Hz

IRNUC : 13C
IRFRQ : 150.80 MHz
IRSET : 125633.89 Hz
IRATN : 511
IRAPW : 65.0 usec
IRBP1 : 50
IRBP2 : 6
IRANS : 0

ADBIT : 16
CTEMP : 30.0 c
CSPED : 0 Hz
SLVNT : C5D5N

RESOL : 11.47 Hz
CLRSO : 97.66 Hz
TLINE : 24
THTOP : 5.0000
THBTM : 0.0100
operator

Fig.14

Elution of the column with ethyl acetate gave a granular compound **GL-5** crystallized from pyridine - benzene - methanol, melting at 298 - 300°C. The homogeniety was checked by thin layer chromatography (silica gel, ethyl acetate - methanol, 9:1) and was detected by iodine and perchloric acid. The IR spectrum of **GL-5** showed a broad band at 3350 cm^{-1} (-OH group). The ^1H NMR spectrum (600 MHz, $CDCl_3$, Fig. 8 a, b) indicated it to be sterol glycoside. The integration of protons and examination of ^1H NMR values in the upfield region of the spectrum suggest it to be mixture of two sterol glycosides. The ^{13}C NMR spectrum (150.8 MHz, C_5D_5N, Fig. 9) showed 63 signals confirming the presence of two glycosides. The comparison of ^1H and ^{13}C NMR spectral values of sitosterol -3-O-β-D- glucoside[11] (Fig. 9, Table I) with that of **GL-5** and assigning the remaining ^1H & ^{13}C NMR signals due to **GL-5** to the other compound, **GL-5** could be characterized as a mixture of Δ^5 and $\Delta^{5,22}$ **sterol-β-D-glucoside** in the ratio of 60 : 40 as determined by integration of protons signal at δ 5.210 due to stigmasterol skeleton and δ 5.331 due to β-sitosterol skeleton. The DEPT experiments (150.8 MHz, C_5D_5N, Fig. 10a-d), HHCOSY (150.8 MHz, C_5D_5N, Fig.11), CHCOSY (150.8 MHz, C_5D_5N, Fig. 12), COLOC (150.8 MHZ, C_5D_5N, Fig. 13), and HMBC (150.8 MHz, C_5D_5N, Fig. 14) studies further support the assignments.

EXPERIMENTAL

The dried roots (2 kg) of *G. lanuginosa* Benth. (Asteraceae) crushed to powder and extracted with methanol. Repeated extractions were made until the solution become colourless. The methanol extracts were dried under reduced pressure. A dark brown mass (130 g) was obtained and treated with chloroform.

The chloroform fraction (45 g) was adsorbed on silica gel (50 g) and transferred to a column of silica gel (150 g) set with petroleum ether (60-80°C). The column was eluted with petrol, petrol-benzene (1:1), benzene, benzene-ethyl acetate (1:1) and ethyl acetate respectively.

The elution of column with petrol and on crystallization afforded compounds **GL-1** and **GL-2**.

GL-1 : Colourless crystalline compound which was crystallized from benzene-chloroform (8:2) having m.p. 157-58° C. Rf = 0.8625 (TLC, SiO_2, petrol-benzene, 1:1), UV inactive, developed by iodine and perchloric acid and soluble in chloroform.

IR υ_{max}^{KBr} cm^{-1} : 3400-3200 (br), 2900, 2850, 1720, 1480, 1460, 1370, 1360, 1240, 1040, 1010, 950.

^{1}H NMR of **GL-1** (mixture of β-sitosterol and stigmasterol) is given in Fig. 1.

GL-2 : Colourless crystalline compound, crystallized in benzene and having melting point 239° C. Soluble in chloroform, developed by iodine and perchloric acid, UV inactive. Rf = 0.2595 (TLC, SiO_2, petrol-benzene, 1:1).

IR υ_{max}^{KBr} cm^{-1} : 2900, 2850, 1720, 1470, 1440, 1370, 1250, 980, 960, 950.

^{1}H MR of **GL-2** (hopane like pentacyclic triterpenoid) is given in Fig. 2.

Elution of column with petrol-benzene (1:1) afforded compounds **GL-3** and **GL-4**.

GL-3 : Colourless crystalline compound, crystallized in benzene, soluble in pyridine and melts at 314° C. Rf = 0.839 (TLC, SiO_2, petrol-benzene, 1:1), UV inactive, developed by iodine and perchloric acid.

IR υ_{max}^{KBr} cm^{-1} : 3450, 2950, 2850, 1720, 1640, 1470, 1440, 1370, 1340, 1240, 1160, 1140, 1100, 1020, 990, 970, 930, 900, 860, 810, 690.

Acetate of GL- 3 : A monoacetate was prepared by treatment of **GL-3** (30 mg) with pyridine (1 ml) and acetic anhydride (1 ml) and having melting point 302-4° C. **^{1}H NMR** of GL-3 acetate is given in Fig. 3.

GL -4 : Colourless, granular compound, crystallized in benzene, chloroform soluble and melts at 89° C. Rf = 0.7111 (TLC, SiO_2, benzene-acetone-glacial acetic acid, 7:3:1). UV inactive developed by iodine and perchloric acid.

Spectral data of GL - 4.

IR υ_{max}^{KBr} cm^{-1} : 2900, 2800, 2300, 1700, 1650, 1460, 1430, 1290, 1200, 930, 870, 820.

MS m/z (rel. int.) : 452 $[M]^+$(4), 428 (48), 396 (10), 368 (20), 139 (30), 83 (30), 74 (66), 57 (100).

^{1}H NMR (600 MHz, $CDCl_3$) : δ 0.867 (t, J=7 Hz, CH_3), 1.321-1.149 (m, 55H), 1.596-1.645 (m, 2H, CH_2), 2.326 (t, 2H, CH_2).

^{13}C NMR data is given in discussion.

Elution of the column with benzene gave inseparable mixture of phenolic

compounds and finally elution of the column with ethyl acetate yielded **GL-5**.

GL -5 : Granular, dirty-coloured compound, crystallized in pyridine-benzene-methanol (1:11:10) and having melting point 298-300° C. Rf = 0.5121 (TLC, SiO_2, ethyl acetate-methanol, 9:1), UV inactive developed by iodine and perchloric acid.

IR υ_{max}^{KBr} cm^{-1} : 3350, 2900, 2850, 1460, 1430, 1380, 1360, 1330, 1150, 1100, 1050, 1010.

From IR, ^1H NMR and ^{13}C NMR data the compound was found to be mixture of Δ^5 and $\Delta^{5,22}$ **sterol-β-D-glucoside**.

REFERENCES

1. Bohlmann, F.; Zdero, C. and Franke, H. *Chem. Ber.* **1973**, 106 (2), 382.

2. Bohlmann, F. and Grenz, M. *Chem. Ber.* **1975**, 108 (1), 26.

3. Bohlmann, F. ; Zdero, C. and LeVan, N. *Phytochemistry* **1979**, 18 (1), 99.

4. Sengupta, P.; Sen, M.; Karuri, P.; Wenkert, E. and Halls, T.D.J. *J. Indian Chem. Soc.* **1985**, 62(11), 916.

5. Guha, P.K. and Bhattacharyya, A. *Indian J. Chem.* **1991**, 30B(7), 714.

6. Nagumo, S. ; Imamura, K.and Inoue, T. *Chem.Pharm. Bull.* **1985**, 33 (11), 4803.

7. Sen, M.; Dey, M. and Karuri, P. *J. Indian Chem. Soc.* **1979**, 56(3), 326.

8. Gu, L.H.; Wang, S.; Li, X. and Zhu, T.R. Yaoxue Xuebao 1987, 22 (4), 272.

9. Gu, L.H. ; Li, X. ;Yan, S.Q. and Zhu, T.R. Yaoxue Xuebao 1989, 24 (10), 744.

10. *The Wealth of India, Raw Material*, CSIR Publication, New Delhi, Vol. IV, **1956**, p.127.

11. Chaurasia, N. and Wichtl, M. *J. Nat. Prod.* **1987**, 50(5), 881.

CHAPTER - IV

POTENTIAL ANTIFILARIAL ACTIVITY OF THE LEAVES OF *MALLOTUS PHILIPPENSIS*, *SENCIO NUDICAULIS* AND THE ROOTS OF *ASPARAGUS ADSCENDENS*, *SAXIFRAGA STRACHEYI* AGAINST *SETARIA CERVI* (NEMATODA : FILARIOIDEA) *IN VITRO*.

INTRODUCTION

Filarial nematode are arthropod borne parasites, which often cause serious diseases in man and animals. The order "filariae" contains four families: Filaridea, Stephanofilaridea, Depetalonematidae, Setariodiae. The adult filariae, of both sex inhabit either the lymphatics, the subcutaneous tissues or mesentary of man. They produce large number of embryos or microfilarae which find their way into the blood stream where they may live for a considerable time without developing further. Their life cycle is continued when the microfilariae are sucked into the stomach of certain blood sucking flies or mosquitos, and completed

Table I. Human filarial infections

Organism	Vectors	Disease
Wuchereria bancrofti	Mosquito (Culex)	Lymphatic filariasis
Brugia malayi	Mosquito (Mansonia)	Lymphatic filariasis
Brugia timori	Mosquito (Anopheles)	Lymphatic filariasis
Onchocerca volvulus	Simulum flies	Subcutaneous nodules and river blindness
Loa loa	Chrysopus flies	Subcutaneous nodules
Dipetalonema perstans	Culicoides	
Dipetalonema streptocerca	Culicoides	Rarely any clinical illness
Mansonella ozzardi	Culicoides	

when they return after further development to the tissues of man during subsequent bites by the insect. The lymphatic filariasis is commonly produced by

Wuchereria bancrofti and *Brugia malayi*. *Loa loa* causes loaisis. *Onchocerca volvulus* produce onchocerciasis which causes river blindness and severe filarial infection (Table 1). Bancroftian filariasis is found throughout the tropical world, while onchocerciasis is limited to Africa, Central and South America. The third serious pathogen of man is *B. malayi* and its distribution is limited to Asia. In India two types of filarial infections, viz. *W. bancrofti* and *B. malayi* occur. Bancroftian filariasis is widely distributed and is responsible for 98% of infections. Brugian filariasis has a localised and restricted distribution (Table II).

Table II. Prevalence of filariasis in various states of India

STATES	PERCENT POPULATION		
	At risk	Mf. carriers	Diseasesd
A.P.	75.4	5.9	2.0
Assam	39.4	1.5	0.35
Bihar	53.1	4.65	6.3
Gujrat	40.7	2.76	0.3
Kerala	100.0	7.8	7.5
M.P.	34.1	1.0	0.1
Maharashtra	18.6	1.2	0.2
Karnataka	24.0	1.6	0.16
Orissa	77.8	7.3	4.6
Tamilnadu	60.3	4.0	2.0
Uttar Pradesh	66.7	5.1	5.2
West Bengal	28.8	1.3	0.03

Haryana, Himachal Pradesh, J & K, Punjab, Rajasthan - N.A.

I. NEED FOR SCREENING :

The only anthelmintic which has been used to treat *B. malayi* in man is diethylcarbamazine (DEC). DEC must be given as a long course of treatment and experience obtained in the South Pacific in attempts to eliminate *W. bancrofti* by mass chemotherapy indicates that large number of people fail to complete the course. The more complicated the treatment and worse the adverse side - effects, the greater the drop out rate. DEC produces much more side-effects in the treatment of *B. malayi* than it does in *W. bancrofti* infection[1]. DEC is far from ideal and a new drug is needed. If the drug which replaced DEC were only microfilaricidal, most of the side effects seen after treatment of brugian filariasis with DEC would be avoided.

Ivermectin a semisynthetic macrocyclic lactone derived from actinomycetes, *Streptomyces avermectis* found to be highly effective against *O. volvulus* is better tolerated safer and more effective microfilaricidal agent than DEC.

Suramin, which kills adult worms and microfilariae, is used in the treatment of onchocerciasis but it is generally considered to be too toxic for use in the treatment of brugian filariasis. Besides this, a number of other drugs kill adult *B. pahangi* in experimental systems but most of these are also too toxic to be used in human patients.

Very few pharmaceutical companies are conducting research on the chemotherapy of filariasis and obtaining compounds for evaluation in experimental filarial screens has been difficult. Experimental drugs are usually available in very small quantities while most tests for filaricidal activity are prodigal in their use of compound. This problem can be overcome by limiting filarial

screens which require minimal amount of drug.

II. SCREENING METHODS AVAILABLE *IN VITRO* AND *IN VIVO* :

1. *In vitro* screening :

Three stages of the life cycle of *L. carinii* have been used for an *in vitro* screen[2].

(a) Adult male and female worms are recovered from the pleural cavity of jirds. After washing, for control cultures, worms are transferred individually to sterile, clear polythene tubes containing 1 ml of Medium 199 plus 10% horse serum. For test purpose worms are placed in various concentrations of the compounds under investigation. The tubes are sealed, maintained at 37° C and observed at frequent intervals using an inverted microscope.

(b) Third-stage infective larvae obtained from *Aedes aegypti* are washed several times in Medium 199 plus antibiotics, placed in each well of microtitre tray containing culture medium or test compounds in various concentrations,. The trays are covered to prevent evaporation and the cultures maintained and observed in the same way as those of adult worms.

(c) Microfilariae are washed from the blood circulation of jirds incubated by this route. A drop of concentrated microfilarial suspension is placed in each well of microtitre tray and culture medium or various concentration of test compound added. The cultures are maintained and observed as above.

The worms survive remarkably well considering the simple media

being used. Long term observations on worms in test compound cultures are not necessary. Control cultures of adult and third stage worms in polythene tubes are viable for at least a week. These techniques were used to study levamisole,also a group of compounds of known filaricidal activity in animals or man and a group of nematodicidal compounds in the *in vitro* systems apart from DEC and Suramin[3].

Thus the value of *in vitro* tests is limited to :

(i) Elucidation of the mode of action of compounds known to be active *in vivo*.

(ii) Comparison of many compounds of the same chemical type.

2. *In vivo* screening (Table III & IV) :

Whatever *in vitro* screens are used, active compounds must be tested in animal models.

Table III. Parasites used for screening potential antifilarial agents *in vivo*

Parasite	Host	Int. Host	Incubation period (days)
L. carinii	Cotton rat	Mite	50
D. viteae	Mastomys/gebril	Mite	42-65
*B. malayi**	Mastomys/gebril	Mosquito	
*B. pahangi**	Mastomys/gebril	Mosquito	59-83
*S. cervi**	rat	Not required	7-13

* Parasites adapted to laboratory animals.

Table IV. Test models for secondary antifilarial screening

Species	Host	Int. Host
B. malayi	Dog, cat	Mosquito
B. pahangi	Monkey	Mosquito
D. viteae	Mastomys/jird	Tick
Dirofilaria immitis	Dog	Mosquito
L. loa	Mandril	Flies
S. cervi	Dog	Not required intra-peritoneal implantation

Filarial infections are closely specific in these hosts and human infections (with partial exception of *Brugia* species) can not be transferred to laboratory animals.

(a) *Litomosoides carinii* :

To date systematic screening tests have been done almost entirely on *Litomosoides* in cotton rats. This infection was used for chemotherapeutic tests[4] and further exploited[5,6] which led to discovery of diethylcarbamazine (Hetrazan). Cotton rats infected with *Litomosoides* provide a practical standardizable test giving an indication of the in vivo action of compound against the filarial worm *Litomosoides*[4,5]. How far *Litomosoides* is a true indication of the chemotherapeutic responses of the human worms *Wuchereria*, *Loa loa* and *Onchocerca* is a matter of question. *Litomosoides* respond well to cyanine, while *Wuchereria* does not; *Litomosoides* does not react to suramin which has a valuable

filaricidal action on *Onchocerca*. Any way, this test is much reliable practical procedure available to date.

(b) *Dipetalonema viteae* :

Jirds infections with *D. viteae* has also been suggested for screening possible antifilarial compounds but this infection is less suitable than *Litomosoides* because (a) jirds are less easy to breed and the infection is less easy to transmit; (b) the adult worms which are under the skin are less easy to remove; (c) the action of drugs upon the microfilariae is difficult to interpret since the number of microfilariae in the blood is low and inconsistent; (d) the chemotherapeutic correspondence to human infections in less close than with *Litomosoides*.

(c) *Brugia pahangi* :

It was found that DEC killed adult worms but not microfilariae of *B. pahangi* and *B. malayi in* cats[7]. Levamisole is a very good microfilaricide against *Brugia* in cats[3,8] and this seems to have action on *B. malayi* in man[9].

III. *Setaria cervi* (Use in screening) :

S. cervi (Nematoda - Filarioidea) is a cosmopolitan parasite in the peritoneal cavity of cattle. It bears a close similarity to human filarial worm in response to drugs. The development and life cycle of *S. cervi* is however not known completely. The adults in peritoneal cavity are non pathogenic. Larvae of *S. cervi* may enter the central nervous system of sheep, goat and horse causing cerebrospinal lesions. In experimental infection in rat, *S. cervi* produces pathological lesions in liver, heart and peritoneal wall etc.

Some successful attempts were made to transplant these worms in rab-

bits[5,10], monkeys[10] and peritoneal cavity of white rats[11,12]. The worms produce microfilariae which were recovered from the peripheral circulation in fairly good number upto a maximum period of six to seven weeks. The earliest recovery of microfilariae was made within 32 h of infection.

IV. CHEMOTHERAPY OF FILARIAL PARASITES :

Hawking[13] attempted to find some chemotherapeutic agents which can influence *W. bancrofti*. Of these unsuccessful attempts and extensive studies only positive results which was obtained till date is that *D. immitis* in dogs can be killed by antimony compounds of fouadin or stibsol type. Hawking tried to find out the filaricidal activity, if any of the different compounds like - Fouadin (antimonial compound), Amthiomalin (arsenic compound), Espundol but no filaricidal action could be demonstrated.

ANTIMONIALS : Among the antimonial, intravenous administration of tartar emetic showed significant reduction in the number of microfilariae of *W. bancrofti*[3]. A positive result had found out on *D. immitis* in dogs where they were killed by antimonial compounds fouadin or stibsol when given in toxic doses but this failed to show significant activity against the adult worms[14]. Other antimonials were shown to be active in different degrees against filariasis. Sdt 779 was found to be most active and thereby regarded as the best antimonial for the treatment of filariasis. Then comes methylglucamine stibonate. Anthiomaline, solustibosan, neostibosan, neostam and fouadin were also used. Fouadin when administered to filarial patients with relatively low dose, severe reactions were elicited. So this type of drugs were not useful. However, solustibosan did not produce unpleasant reactions in patients and also was not very effective[15]. Pentosam and methylglucamine stibonate

can be given to man for radical cure in such a high dose which will not produce serious side effects. As the drugs are excreted rapidly doses are to be given frequently.

ARSENICALS : Compounds of arsenic were also used for the study of antifilarial action of which very few like a derivative of p- benzamide arsenoxide D-324, neoarsphenamine and a trivalent derivative of tryparsamide possess activity against adult worms. MEL W. (Melarsonil potassium) was found to be of extensive use in the treatment of *O. Volvulus*[16] or *W. bancrofti*[16] where there is reduction in the number of microfilariae and found that it was very potent drug in terms of complete immobilization of worms[17].

CYANINE QUINOLINE GROUP AND PHENANTHRIDIUM COMPOUNDS : High degree of acitivty with good results were seen in styryl, anti- quinoline and benzothiazoles[18]. The filaricidal action of phenosafranine series which was tested upon cotton rats infected with *L. carinii,* killed adult worms but did not affect the microfilariae *in vivo*[19]. Methylene voilet had no filaricidal action upon the microfilariae or adult worms of *W. bancrofti* or *A. perstans*, so also in *O. Volvulus.* Thus phenosafranine series have hardly any value for the treatment of human filariasis.

PIPERAZINE : The compound 18C (1-carbethoxy-4-methyl piperazine hydrochloride) was effective in reducing the microfilarial content in the filaria infected cotton rats[20]. In cotton rats, treated with several piperazines, autopsies revealed no dead adult worm was present in pleural cavity but dramatic reduction in the number of microfilariae. Effect of piperazine against *D. immitis* in the dog was also observed. As compared to antimony derivatives, piperazines produced a much more rapid effect upon the microfilariae of cotton rats and when

compared to antimony salts piperazine showed slowed effect against adult worms.[15]

DIETHYLCARBAMAZINE (DEC) :- Hetrazan (1-diethylcarbamyl -4- methyl piperazine) rapidly reduced the microfilariae of *L. carinii* in cotton rats and *W. bancrofti* in human beings[21]. 1-diethylcarbamyl -1, 4- methyl piperazine hydrochloride was the most promising agent. After oral or intraperitoneal treatment with this compound in cotton rats infected with filaria (dose being 3-100 mg / kg) microfilarial count drops within 24h. There may or may not be reappearance of microfilariae in the peripheral blood on cessation of treatment. Similar effect, but lesser to some extent occurs with the microfilariae of *L. Carinii*[20]. However,the effect of drug on adult filarial worms are not demonstrable so quickly like microfilaria.Lethal effect on the adult worms depend upon various important factors : (i) Amount of drug given; (ii) Frequency of dosage; (iii) The number of days elapsing from cessation of treatment to autopsy; (iv) Methylpiperazine hydrochloride in a dose of 10 mg / kg or more, twice or more daily, 72% showed death of either some or all adults or no adult worms.But the dose was maintained from 3.13 to 100 mg/kg twice. The rats were treated with both oral and intraperitoneal treatment because there was no significant difference in the effects of either routes of administration.

IVERMECTIN : Apart from diethylcarbamazine, ivermectin was also found to have some satisfactory antifilarial effect. Ivermectin is a semisynthetic macrocyclic lactone, which is derived from actinomycetes, *S. avermactis*, highly effective against a broad range of helminthic parasites and arthropods[22]. Experience in treating human being with this drug in *O.Volvulus* infection was found to be microfilaricidal with minimal evidence of toxicity. Even in a very low

doses 5 to 50 μg/kg, ivermectin caused miicrofilaricidal effect [23,24]. In patients with onchocerciosis, a single dose of 150 μg/kg, of ivermectin repeated once a year leads to a marked reduction in skin microfilariae counts and ocular involvement[25]. At this dose ivermectin causes minimal side effects and is sufficiently free of severe reactions to be used on a mass scale. It was compared with DEC for the patients received single oral dose (200 μg/kg, body weight) of ivermectin and DEC daily for eight days or placebo. Analysis showed that ivermectin was better tolerated, safer and more effective microfilaricidal agent than DEC for the treatment of onchocerciasis[26]. Given the practical advantages of single dose administration, ivermectin should become a useful medication for the control of bancroftain filariasis.

NATURAL PRODUCTS : Crude extract of the stem bark of plant *Streblus asper* killed microfilariae as well as adult *L. carnii.* This effect was found to be due to two cardiotonic glycosides, asperoxide and strebloside. The former also showed strong activity against *B. malayi* in mastomys. In order to dissociate the cardiotonic activity from antifilarial potential, various chemical transformations of these glycosides were carried out. The aqueous and alcoholic extracts of *S. asper* showed potential antifilarial activity against *S. cervi in vitro* at very low concentration[27],

In forty nine cases, legs (68) with filarial elephantasis at the lower extremities were treated with the extracts from mulberry leaves by intramuscular injection of 5 ml per day for 7 successive courses each lasting 21 days at the interval of 9 to 10 days and the effective rate was 100% . The nearby curative rates at each phase from one to seven course were 8.82%, 19.41%, 23.88%, 32.82%, 39.07%, 48.39%, and 55.00% showing that the effect improved with

increase in number of courses[28].

The alcoholic and aqueous extracts of *Argyria speciosa* also have antifilarial activity against *S. cervi in vitro*[29]. The concentration of aqueous extract required to kill 50 & 90% larvae were 2.4 & 3.6 μg/ml respectively. The alcoholic extract caused death of larvae at much lower concentration, the LC_{50} & LC_{90} being only 75 and 125 ng/ml respectively.

OBSERVATIONS

I. *Mallotus Philioppensis*

Alcoholic and etheral extracts of M. *philippensis* (Lam.) Muell. Arg. fruits have shown taenicidal action against *Hymanolipis naua* and *H. diminuta*, both in vitro and in *vivo*. The extracts showed lethal action on trematodes (*Fasciolipsis buski*) but not on nematode *Ascaris lumbricoides in vitro* [30, 31]. *M. philippensis* powder as used in Ayurvedic (indigenous) system of medicine was found effective in conversion of stools from positive to negative in 96% of children harbouring *H. naua.*

Plant powder is used in external application for parasitic infections of skin and also as aphrodisiac, lithontriptic and styphic [32-34]. Kamla powder is also used as an antiseptic in blisters in the ear[35]. Phytochemical studies of Kamla have revealed the presence of a number of prenylated flavonoides [36,37] including rottlerin [37-40], isorottlerin[39], isoallorottlerin[40], 4-hydroxyrottlerin[41], 3,4 - dihydroxyrottlerin[41], triterpenoids[42] (lupeol acetate, lupeol, sitosterol, α-amyrin, acetyl aleuritolic acid) and bergenin[42].

There is no report on anthelmintic activity of *M.philippensis* leaves in literature. Further the plant has not been explored for antifilarial activity. It was, therefore, thought worthwhile to test alcoholic and aqueous extracts of *M.philippensis* for potential antifilarial activity *in vitro*.

Effect of aqueous extract of *M.philiphhensis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : A typical response to aqueous extract of *M. philippensis* on the spontaneous movements of whole worm of *S. cervi* is shown in Fig. 1. Immediately after the addition of

the extract in a concentration of 100 μg/ml to the bath fluid, the amplitude of contractions showed a gradual increase. The amplitude continued to increase till about 60 min the time when it reached its peak. With the increase in amplitude, the rate of contractions showed a corresponding decrease while the tone remained unaffected. Thereafter the spontaneous movements became frequent and the amplitude also showed a gradual decrease. After about 95 min the movements of whole worm ceased completely. Repeated changes of bathing fluid was not beneficial in restoring the movements. Addition of Ach (acetylcholine) at this stage in concentrations of 5 and 100 μg/ml of bath fluid had only a very little response. Addition of KCl to the bath fluid showed a small single twich.

The response to aqueous extract of *M. philippensis* could be elicited on n.m. complex of *S. cervi* in a concentration of 20 μg/ml i.e., 1/5 th of that required for the whole worm. The response too was different in nature. The initial stimulant effect characterized by increase in amplitude of contractions observed with whole worm was not observed with n.m. preparation.The movements were characterized by decrease in amplitude only. The tone and rate of contractions remained visibly unaffected. The depressant effect was reversible as repeated changes of bathing fluid (W), restore the movements to normal.

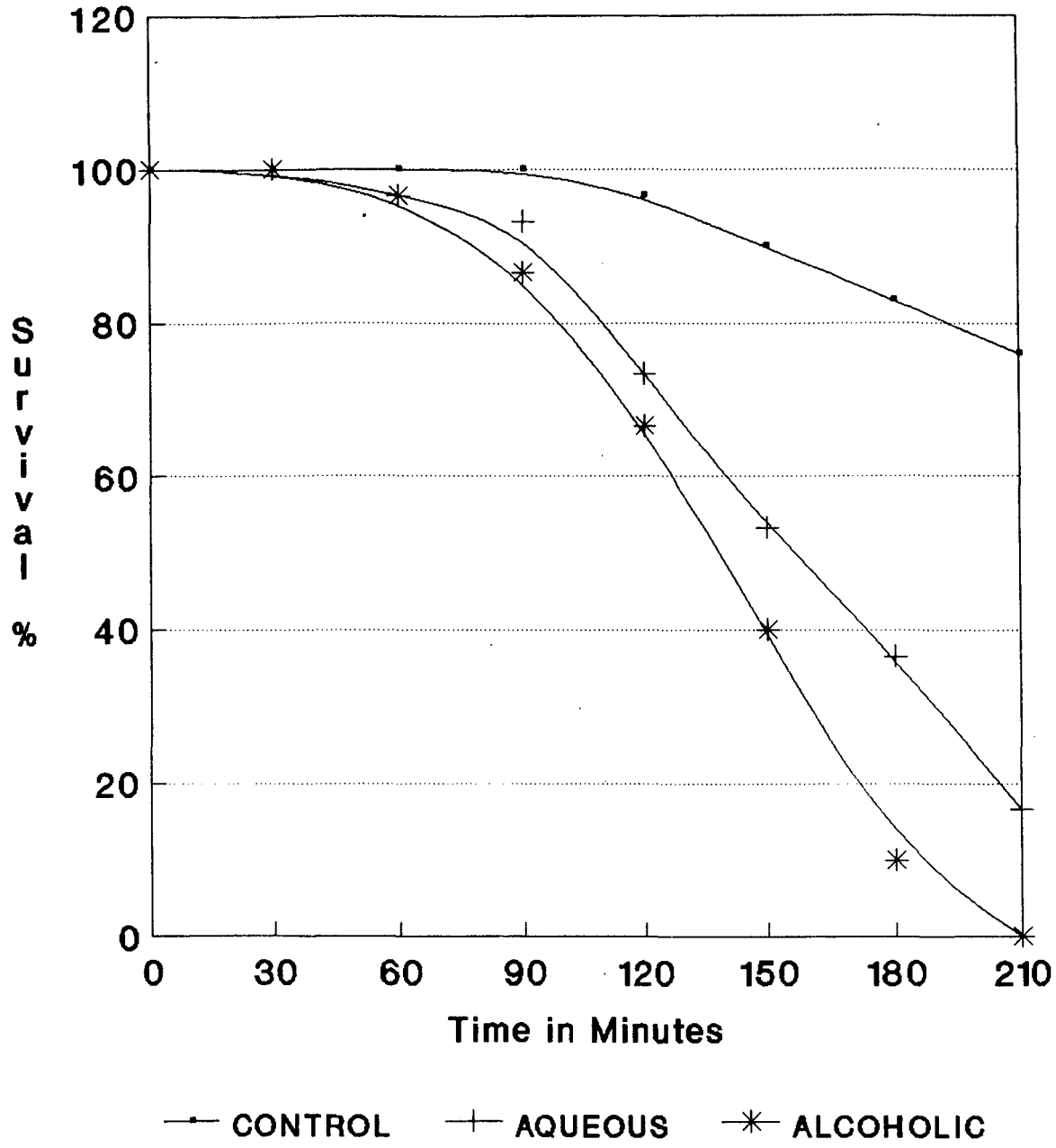
With a higher concentration of aqueous extract (40 μg/ml) on n.m. preparation the response was immediate with reduction in tone characterized by lowering of base line and cessation of movements. The effect at this concentration was irreversible as the washing of the preparation with bathing fluid failed to restore the movements. The n.m. preparation responded with a well defined typical response of Ach (lower panel of Fig.2).

Effect of alcoholic extract of *M. philippensis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*: Addition of alcoholic extract of *M. philippensis* in a concentration of 230 μg/ml of bath fluid caused initial stimulation followed by the paralysis of whole worm of *S. cervi*. The initial stimulant effect which lasted for about 45 min was characterized by shortlasting increase in amplitude. In the later part of stimulant phase i.e., after nearly 30 min the movements became less frequent. This was followed by paralysis of worm characterized by complete cessation of movements, which have not restored spontaneously till about 6h. However, repeated changes of bath fluid restored the movements, showing that the paralysis induced by the alcoholic extract of *M. philippensis* is reversible in nature (Fig.3).

The effect of the alcoholic extract of *M. philippensis* on n.m. preparation of *S. cervi* was manifest in a concentration nearly 10 times less (20 μg/ml) than required for the whole worm preparation. The initial sitmulant effect was characterized by increase in amplitude alone and there was no increase in tone as seen with the whole worm. Further, the stimulant effect lasted for only 5 min as compared to 45 min with whole worm. The paralysis which followed was similar in nature and if the bath fluid was not changed it continued for more than 6 h (time till the preparation was observed). However, repeated changes of the bathing fluid restored the movements to normal (Fig.4).

Aqueous and alcoholic extracts of *M. philippensis* leaves caused concentration related effect on the survival of microfilariae of *S. cervi*. The LC_{50} and LC_{90} as observed after 6 h is presneted in Table V. The alcoholic extract being more potent in its lethal effect as compared to the aqueous extract. Effect of aqueous and alcoholic extracts of *M. philippensis* in a concentration of 25 ng/

Fig.5: **Effect of aqueous and alcoholic extracts of the leaves of Mallotus philippensis on the survival of microfilariae of Setaria cervi in-vitro at a concentration of 25 ng/ml. Abscissa denotes time in minutes and ordinate denotes percentage of survival.**



ml observed for 210 min is shown in Fig.5.

II. *Sencio nudicaulis*

Sencio nudicaulis Buch . Ham . of family compositae is indigenous to temperate Himalaya.Ragwort poisoning due to several species is well known in animals, various species prduced hepatic cirrhosis[34]. Phytochemical studies of *S. nudicaulis* species revealed the presence of alkaloids[43], 3α, 6β, bis (angeloxy) furanoere mophilane and γ-humulene[44]. However, no pharmacological activity has been reported for *S. nudicaulis.*

Effect of aqueous extract of *S. nudicaulis* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : A typical response of aqueous extract of *S. nudicaulis* on whole worm (upper panel) and n.m. preparation (lower panel) of *S. cervi* is shown in Fig. 6. At a concentration of 30 μg/ml the extract caused decreased in the spontaneous movements of whole worm. The depressant effect was characterized by decrease in tone, amplitude and rate of contractions. After about 5 min the worm movements ceased completely. Repeated changes of the bathing fluid failed to restore the movements. The paralysis caused by is therefore irreversible in nature. Addition of Ach in a concentration of 5 μg/ml failed to elicit its typical stimulant effect.

On n.m. preparation the depressant effect was similar in nature but was evident at a lower concentration of 10 μg/ml. The amplitude, tone and rate of contractions showed a steady decline leading to complete paralysis of the n.m. preparation about 10 min after the addition of drug. Repeated changes of the bathing fluid failed to restore the movements till upto 6 h. The preparation however responded to the addition of Ach. The response was in the form of a single spike and the preparation continued to be paralysed thereafter.

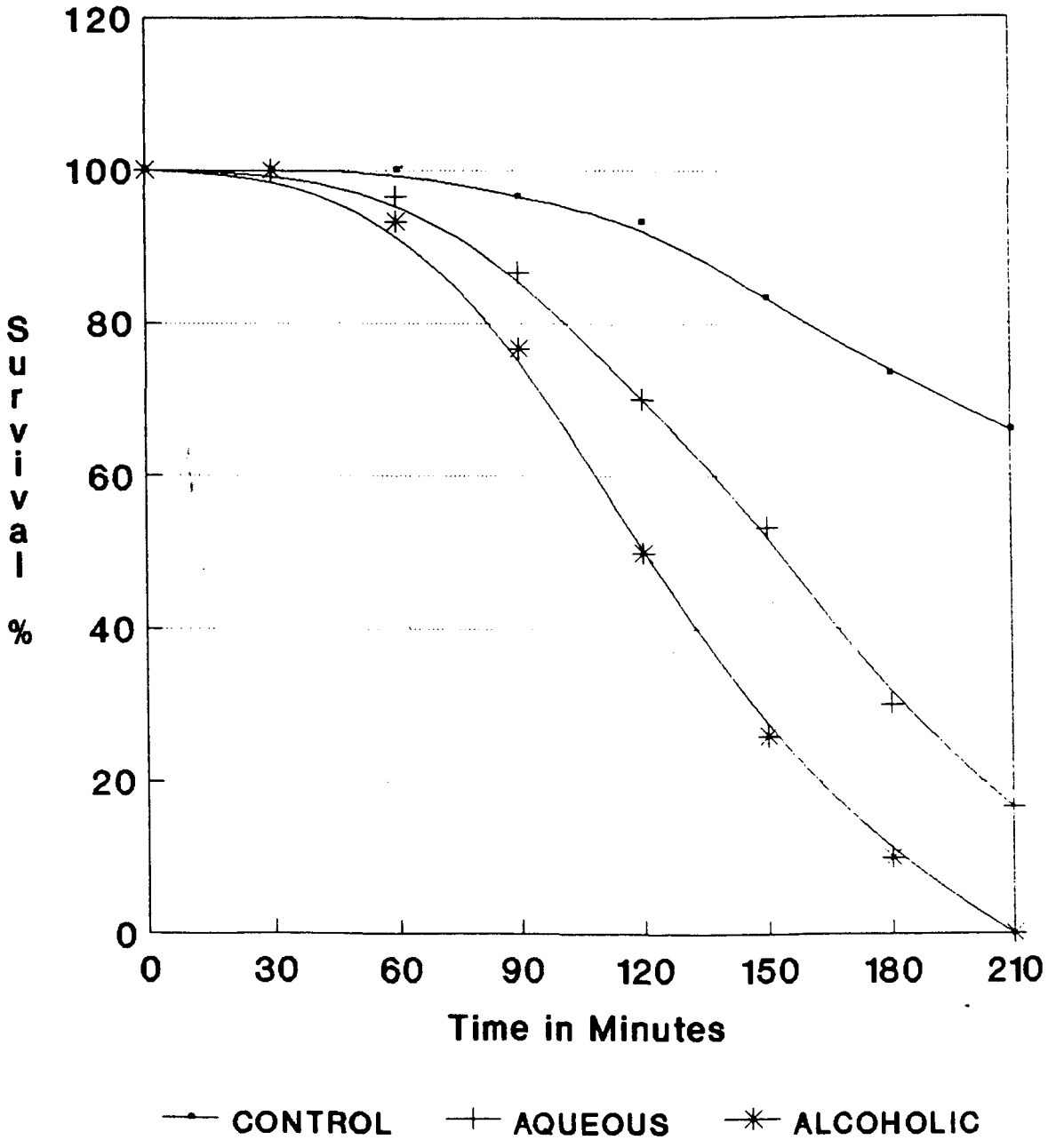
The effect of alcoholic extract of S. nudicaulis on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*.: The response to alcoholic extract of *S. nudicaulis* on whole worm and n.m. preparation was not similar to that observed with the aqueous extract. Addition of alocholic extract of *S. nudicaulis* on the spontaneous movements of whole worm in a concentration of 80 μg/ml to the bath fluid caused initial stimulation characterized by increase in amplitude which lasted nealy for 5 min (Fig. 7). Thereafter the amplitude started showing a decrease but occasional spikes of high amplitude were seen at irregular intervals.After about 60 min these spikes of high amplitude were not seen and the amplitude continued to decrease and in another about 20 min the movements of the worm ceased completely.

Repeated washing with the bathing fluid failed to restore the movements. The paralysis induced was therefore, irreversible.When Ach. was added to the bath fluid at this time a typical response was observed in the form of stimulations lasting about 5 min.

On n. m. preparation the effect was evident in a concentration 20 times less than that required for whole worm preparation.The response was characterized by initial shortlasting (2-3 min) stimulation followed by paralysis. Repeated changes of bath fluid failed to restore the movements of n.m. preparation. However, the stimulant effect of Ach could be elicited following addition of the drug in a concentration of 5 μg/ml of the bath fluid. The movements of n.m. preparation continued to remain paralysed upto 6 h till when the preparation was observed and there was no sign of recovery (Fig.8).

Both exracts of *S.nudicaulis* leaves caused concentration related effect on the survival of microfilariae of *S. cervi*. The LC_{50} and LC_{90} as observed after

Fig.9: **Effect of aqueous and alcoholic extracts of the leaves of Sencio nudicaulis on the survival of microfilariae of Setaria cervi in-vitro at a concentration of 25 ng/ml. Abscissa denotes time in minutes and ordinate denotes percentage of survival.**



6h is presented in Table V. The alcoholic extract being more potent in its lethal effect as compared to the aqueous extract. Effect of aqueous and alcoholic extracts of *S. nudicaulis* in a cencentration of 25 ng/ml observed for 210 min is shown in Fig. 9.

III. *Asparagus adscendens*

Tubers of *Asparagus adscendens* Roxb. (Liliaceae) are used in the indigenous system of medicine, for the treatment of spermatorrhoea, gleet, chronic leucorrhoea, diarrhoea, dysentry and general debility[32,34]. Stems are used as aphrodisiac. As an ingredient of indigenous drug 'Geriforte' it is used for senile pruritus and against fatigue[45].

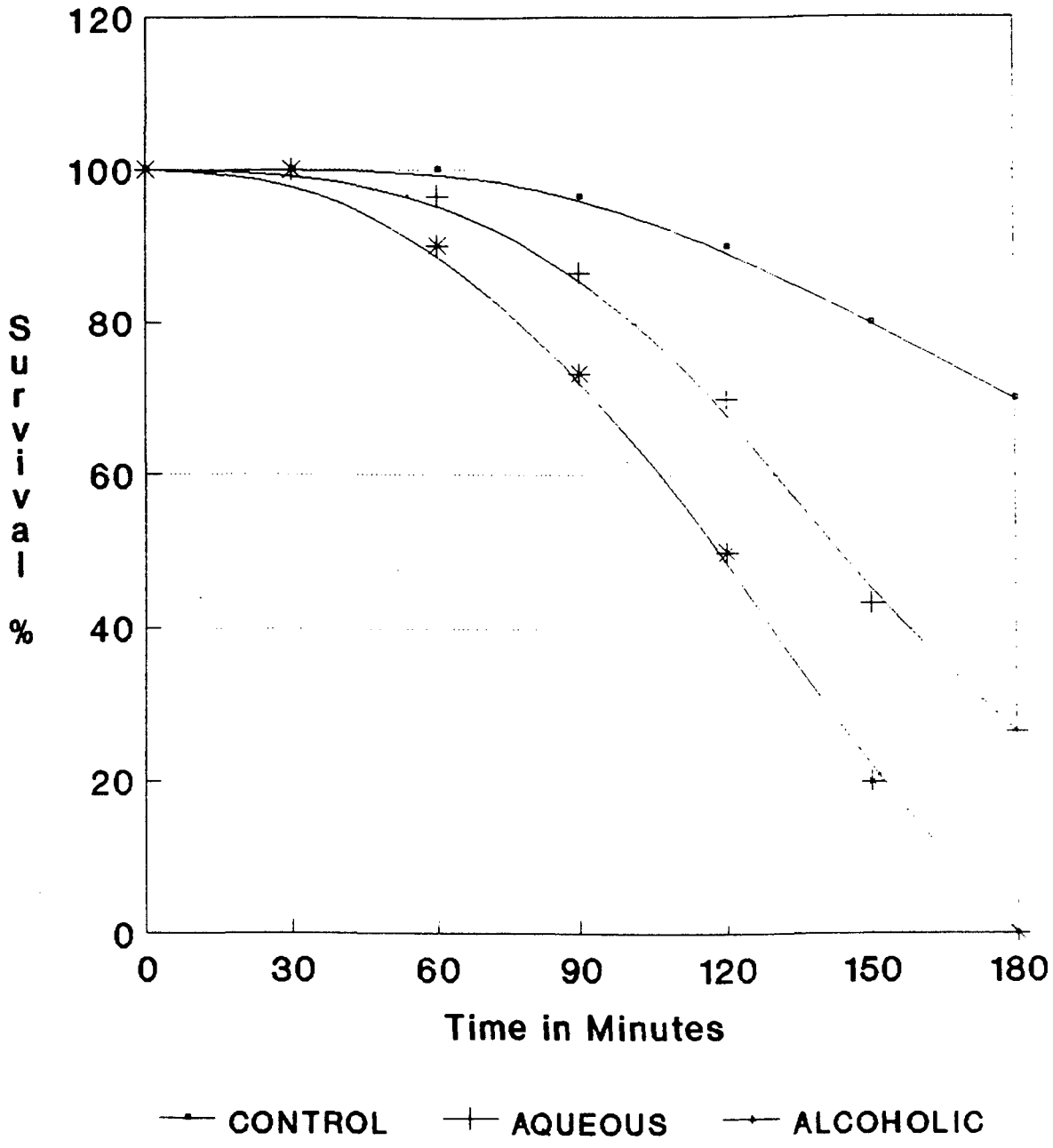
Phytochemical studies of the roots of *A. adscendens* revealed the presence of several sapogenins and saponins.The steroidal glycosides which have been isolated and identified are asparanin -A[46], - B[46], -C[47], and -D[47]; asparoside-A[46], - B[46], -C[47] and- D[47]; adscendin-A[48] and -B[48]; adscendoside-A[48] and-B[48], 3-β-o [β-D-2-tetracosylxylopyranosyl] - stigmasterol[49]. Its fruits also contain β-sitosterol, sarsapogenin and diosgenin[50]. No report appears in the literature suggesting its role as an anthelmintic.

During routine screening, the ability of the root extract to inhibit the spontaneous motility of filarial parasite *S.cervi* generated interest and it was thought worthwhile to explore anthelmintic potential of the two extracts (aqueous and alcoholic).

Effect of aqueous extract of *A. adscendens* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : A typical response of aqueous extract of *A. adscendens* on the spontaneous movements of whole worm of *S. cervi* is shown in Fig. 10. Addition of extract in a concentration of 30 μg/ml to the bath fluid caused an increase in the rate, tone and amplitude of contractions. The effect was evident immediately after the addition of drug. The effect on the tone was shortlived continuing over a few min. The increase in rate and amplitude continued for nearly 3 h after that the movements returned to normal. With a higher concentration of 100 μg/ml the stimulant effect of the drug characterized by shortlasting small increase in tone and amplitude followed by cessation of worm movements. The worm continued to remain paralyse for more than 6 h. However, repeated changes with the bathing fluid, the movements of the worm were restored to normal. This reversible paralysis has been shown in lower right panel of Fig. 10 when the worm was given repeated washing after 60 min.

On n.m. preparation the aqueous extract of *A. adscendens* produce decrease in spontaneous movements characterized by decrease in amplitude, rate and tone of contractions. The initial stimulant effect was not observed. It took about 30 min for a concentration of 30 μg/ml to completely paralyze n.m. preparation. The movements were however not restored despite repeated changes of the bathing fluid (w). This indicates that the paralysis caused was irreversible.There was no indication of restoration of movements even after 6 h. Additon of Ach, an excitatory neurotransmitter to the bath fluid could elicit the response. The response to Ach was concentration related being more with a concentration or 5 μg/ml as compared to 1 μg/ml (Fig. 11).

Fig.13:Effect of aqueous and alcoholic extracts of the roots of Asparagus adscendens on the survival of microfilariae of Setaria cervi in-vitro at a concentration of 25 ng/ml. Abscissa denotes time in minutes and ordinate denotes percentage of survival.



Effect of alcoholic extract of *A. adscendens* on the spontaneous movements of whole worm and n.m. preparation of *S.cervi* : A typical response to the alcoholic extract of *A. adscendens* on whole worm (upper panel) and n.m. preparation (lower panel) is shown in Fig.12 .The response of alcohlic extract was similar in nature to the aqueous extract.Following addition of 75 μg/ml of the alcohlic extract, whole worm showed an initial in creasein spontaneous activities characterized by increase in amplitude and tone of contractions.This effect lasted for about 4-5 min and was followed by decrease in amplitude, rate and toneof contractions, and the movements ceased completely after about 10 min. The paralysis of the worm thus caused continued for more than 6 h and there was no spontaneous recovery. However after repeated changes of the bathing fluid (w), restored the movements to pre drug level.

On n.m. preparation the effect of alcoholic extract of *A. adscendens* was similar in nature to that observed with whole worm. However the concentration required to produce an equivalent effect was only 30 μg/ml as compared 75 μg/ml for the whole worm. The paralysis caused was reversible too as the repeated washing restored the movements.

Both aqueous and alcoholic extracts of the roots of *A. adscendens* caused concentration related effect on the survival of microfilariae of *S. cervi*. The LC_{50} and LC_{90} observed after 6h is presented in Table V. The alcoholic extract being more potent in its lethal effect as compared to the aqueous extract. Effect of aqueous and alcoholic extracts of *A. adscendens* in a concentration of 25 ng/ml observed for 180 min is shown in Fig. 13.

IV. *Saxifraga stracheyi*

Root extract of *Saxifraga stracheyi* has been used in Ayurvedic system of medicine for the diseases of urinary tract[45]. Although no reference is available for its use in helminthic or any other parasitic infections, its effectiveness in spasmodic pain of the urinary tract which may result from action on neurotransmitters prompted us to study the effect of aqueous and alcoholic extracts of *S. stracheyi* on the motility and survival of adult worm and the microfilariae of filarial nematode parasite *S. cervi*. Some ingredients including β-sitosterol, (+) catechin -3- gallate and bergenin have been identified in the roots of *S. stracheyi* [51,52]. Since effect of entire extract which is composite response of all the ingredients may be different from an isolated single chemical substance, hence the study was planned with aqueous and alcoholic extracts.

Effect of aqueous extract of *S. stracheyi* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi*: Addtion of 140 μg/ml of aqueous extract of *S. stracheyi* to the bath fluid modified the movements of whole worm of *S. cervi*, while at lower concentrations, it was inactive.The response was characterized by initial stimulation followed by paralysis. The initial stimulatory response was characterized by increase in amplitude while the rate of contraction decreased. There was no visible change in tone. The amplitude continued to exhibit an increase till about 2 h and 30 min whereafter it started declining and in another 30 min contractions ceased completely. Repeated washing with the bathing fluid failed to restore the movements of whole worm (Fig.14).

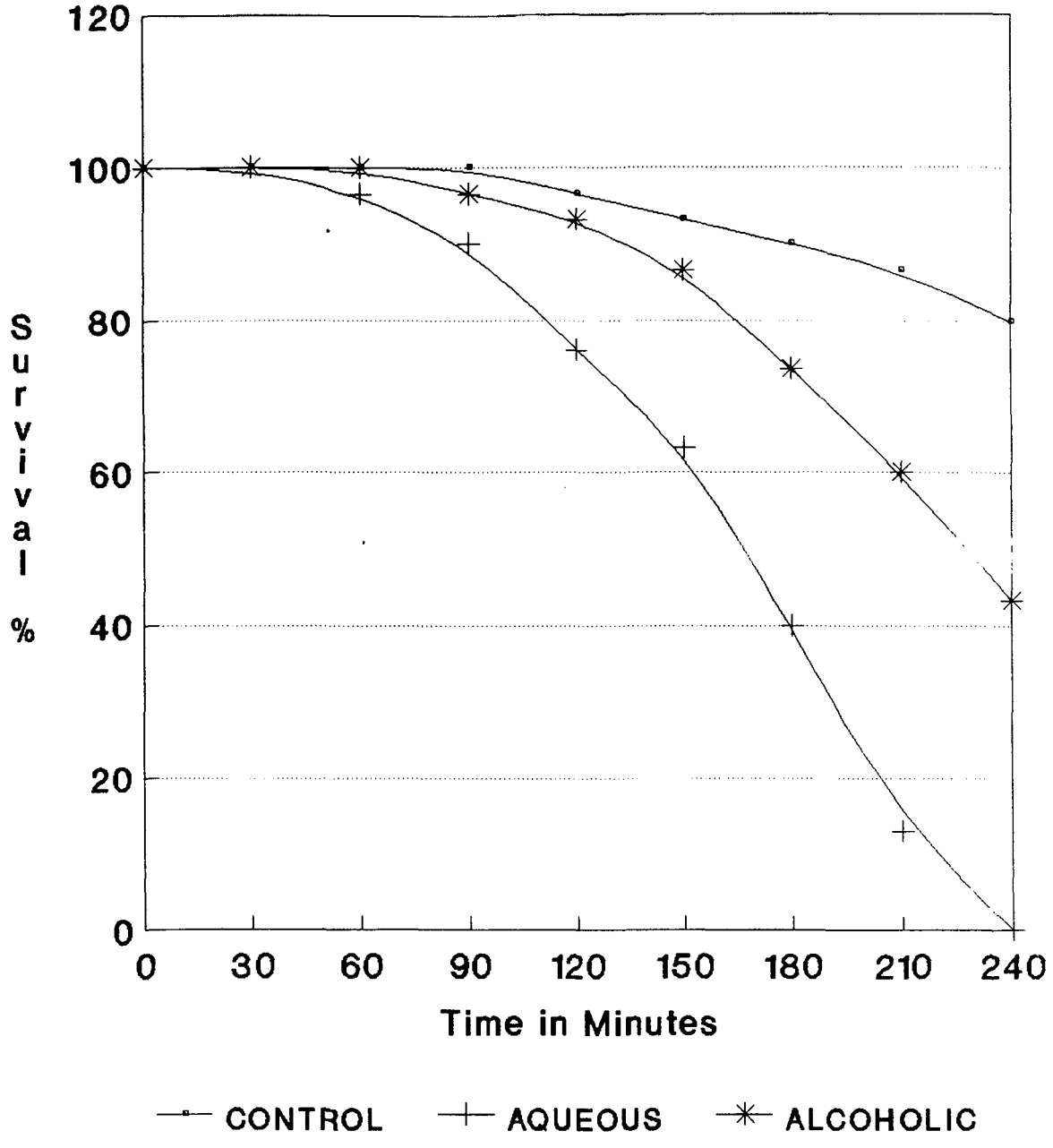
On n.m. preparation the effect of aqueous extract was manifest at a concentration as low as 30 ug/ml of bath fluid. The onset of initial stimulation was

observed after an interval of few minutes (Fig. 15 upper left panel) but became visibly evident after about 15 min of addition of the extract to the bathing fluid. The response was characterized by increase in amplitude while rate and tone of contractions remained nearly unaffected. The phase of stimulation was shortlived and after another 10 min the amplitude started decreasing and became lower than the pre drug level (Fig. 15 upper right panel). The movements though not restored to normal, the worm showed a spurt of shortlived activity which was followed by complete cessation of movements (Fig. 15 lower right and left panel). Repeated washing with drug free bathing fluid could not restore the movements.

Effect of alcoholic extract of *S. stracheyi* on the spontaneous movements of whole worm and n.m. preparation of *S. cervi* : The response to the alcoholic extract of *S. stracheyi* was not quite similar to that observed with the aqueous extract. Addition of alcoholic extract in a concentration of 250 μg/ml caused immediate initial stimulation and was characterized by increase in amplitude and tone of contractions. The rate of contractions was unchanged initially but showed a decrease after about 30 min when the increase in amplitude became highly significant and visible. The rate thereafter started declining slowly while the amplitude continuing to remain increased. After about 90 min decrease in rate of contractions became distant and amplitude decreased. After about 3 h when the preparation was given repeated wash with bathing fluid the contractions were restored to normal (Fig. 16).

The effect on n.m. preparation (Fig. 17) was manifest with a concentration about 14 times less than that required to affect the movements of whole worm preparation. Addition of alcoholic extract in a concentration of 20 μg/ml

Fig.18: **Effect of aqueous and alcoholic extracts of the roots of Saxifraga stracheyi on the survival of microfilariae of Setaria cervi in-vitro at a concentration of 25 ng/ml. Abscissa denotes time in minutes and ordinate denotes percentage of survival.**



caused reduction in rate and amplitude of contractions while tone showing no visible change. Nearly 5 min after the addition of drug, the worm was completely paralysed. Repeated washing with the bathing fluid was effective in restoration of the movements.

Table V. Effect of plant extracts on the survival of microfilariae of *S. cervi in vitro*

Plants	Extract	Lethal Concentrations ng/ml	
		LC_{50}	LC_{90}
M.philippensis	Aqueous	18	20
	Alcoholic	12	15
S. nudicaulis	Aqueous	10	19
	Alcoholic	5	12
A. adscendens	Aqueous	8	16
	Alcoholic	3	12
S. stracheyi	Aqueous	8	20
	Alcoholic	10	23

The LC_{50} and LC_{90} as observed after 6 h is presented in Table V. The aqueous extract being more potent in its lethal effect as compared to alcoholic extract. Effect of aqueous and alcoholic extracts of *S. stracheyi* in a concentration of 25 ng/ml observed for 240 min is shown in Fig. 18.

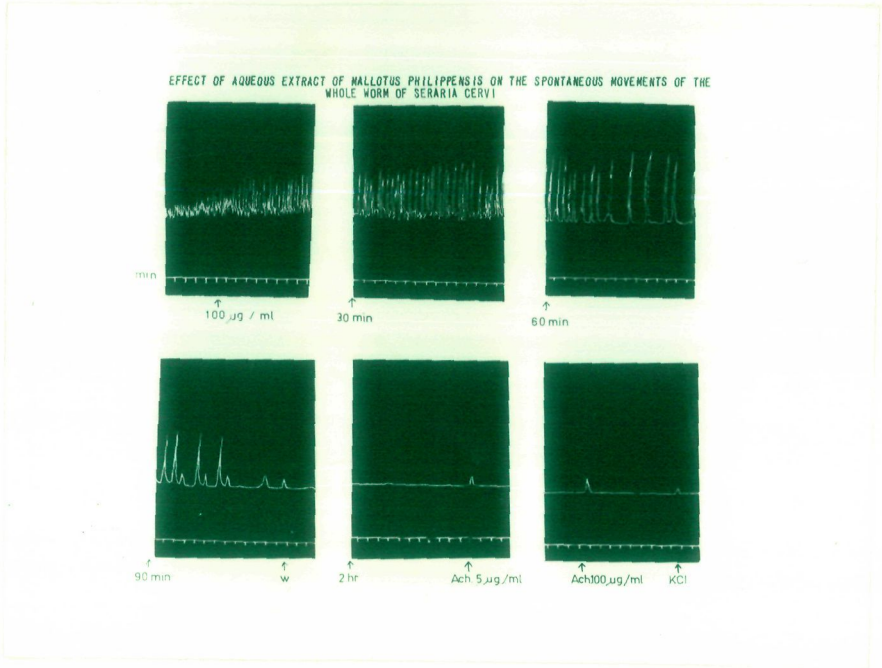


Fig. 1: Effect of aqueous extract of the leaves of *M. philippensis* on the spontaneous movements of whole worm of *S. cervi*. Bath applied concentration of 100 µg/ml caused initial stimulation characterized by increase in amplitude followed by irreversible paralysis. With the increase in amplitude, the rate of contractions showed a corresponding decrease while the tone remained visibly unaffected. The stimulant effect of Ach was blocked by whole worm movements while the effect of KCl was in the form of single small twich.

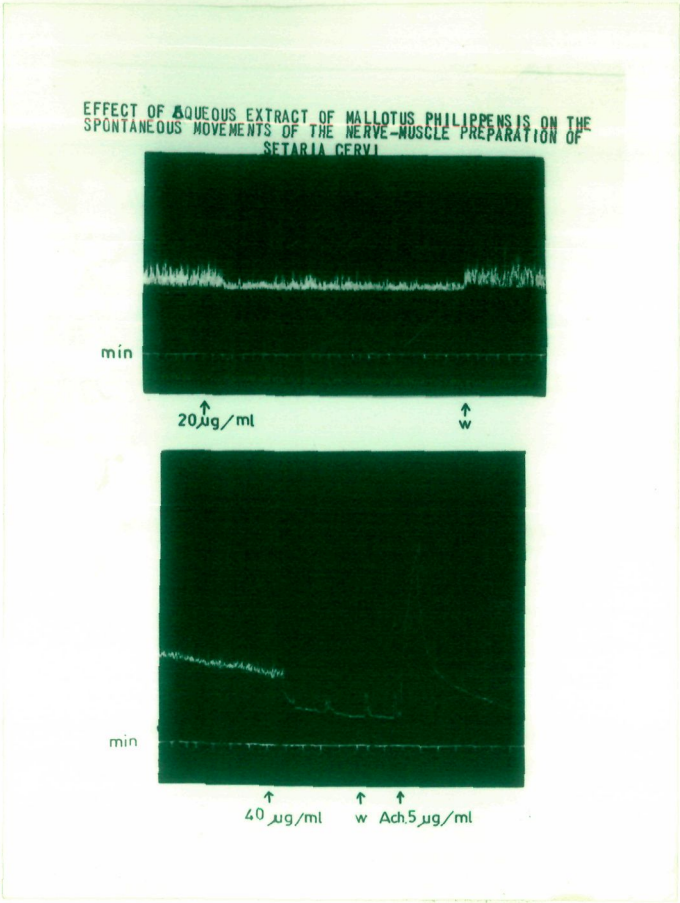


Fig. 2: Effect of auqueous extract of the leaves of *M. philippensis* on the spontaneous movements of n.m. preparation of *S. cervi*. A concentration of 20 µg/ml caused decrease in amplitude and tone while the rate of contractions remained visibly unaffected. But at a higher concentration (40 µg/ml) the paralysis caused was irreversible as repeated washing (w) failed to restore the movement. Ach (5 µg/ml) produced its usual stimulant effect during the phase of paralysis.

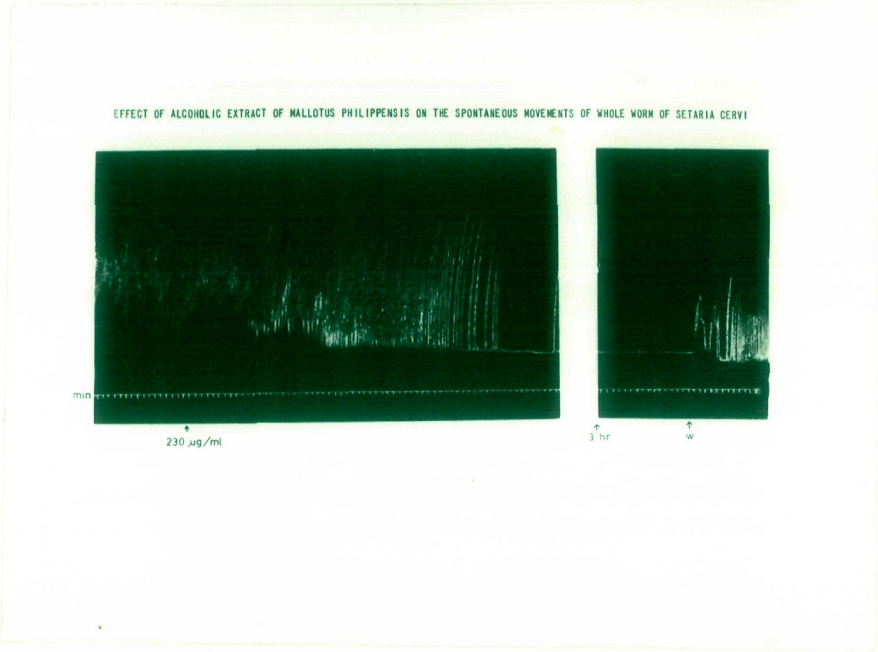


Fig. 3: Effect of alcoholic extract of the leaves of *M. philippensis* on the whole worm of *S.cervi*. Bath applied concentration of 230 µg/ml caused initial stimulation characterized by increase in amplitude (lasting for 2h) followed by reversible paralysis. The tone showed an initial short lasting increase while the rate decreased in the later part of stimulant phase.

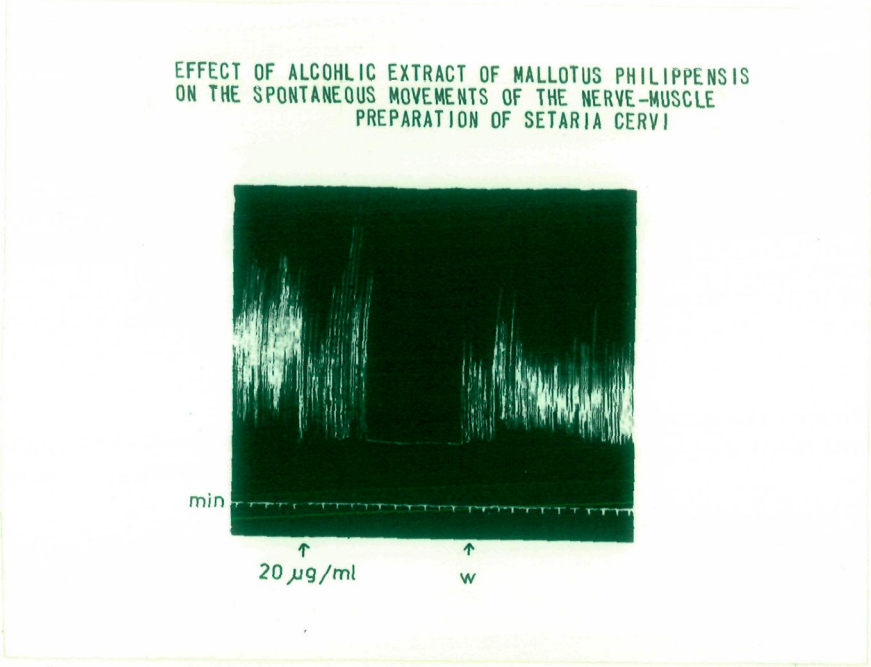


Fig. 4: Effect of alcoholic extract of the leaves of *M. philippensis* on the spontaneous movements of n.m.preparation of *S. Cervi*. Bath applied concentration of 20 µg/ml caused initial stimulation characterized by increase in amplitude followed by reversible paralysis. The rate of contractions remained visibly unaffected while the tone showed a gradual decrease.

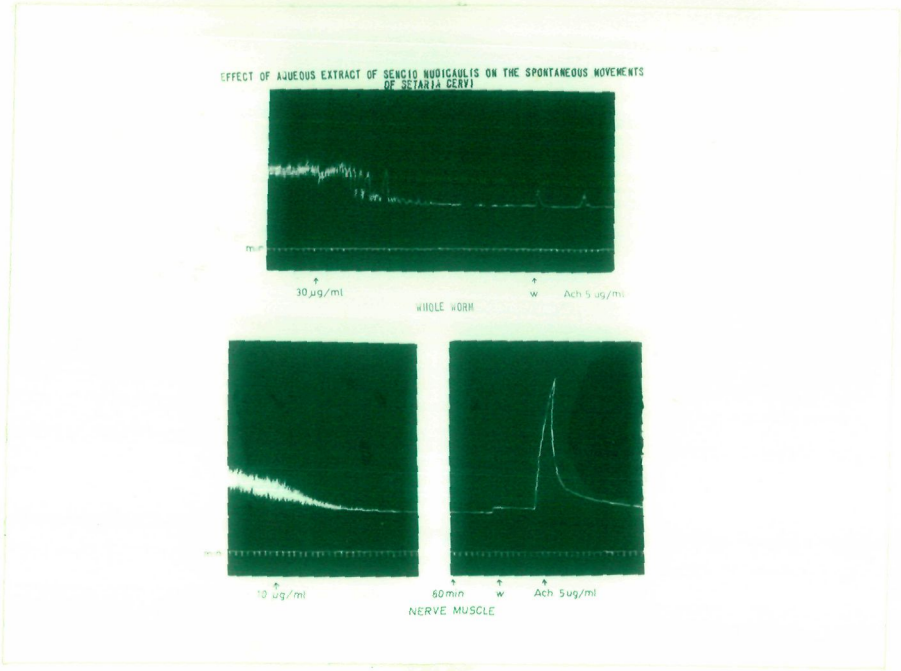


Fig. 6: Effect of aqueous extract of the leaves of *S. nudicaulis* on the spontaneous movements of whole worm (upper panel) and n.m. preparation (lower panel) of *S. cervi*. A concentration of 30 µg/ml produced irreversible paralysis of whole worm while only 10 µg/ml was required to paralyse n.m. preparation. The stimulant effect of Ach was not blocked.

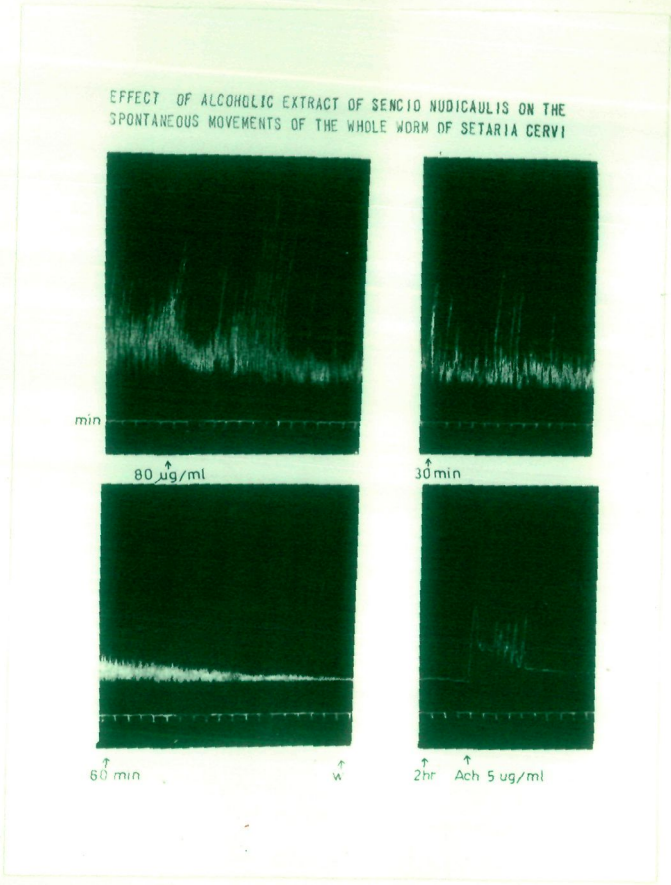


Fig. 7: Effect of alcoholic extract of the leaves of *S. nudicaulis* on the spontaneous movements of whole worm of *S. cervi*. Bath applied concentration of 80 µg/ml caused stimulation characterized by increase in amplitude lasting for about 45 min (upper panels). Thereafter the amplitude started decreasing leading to irreversible paralysis. Response to Ach is seen in the lower right panel.

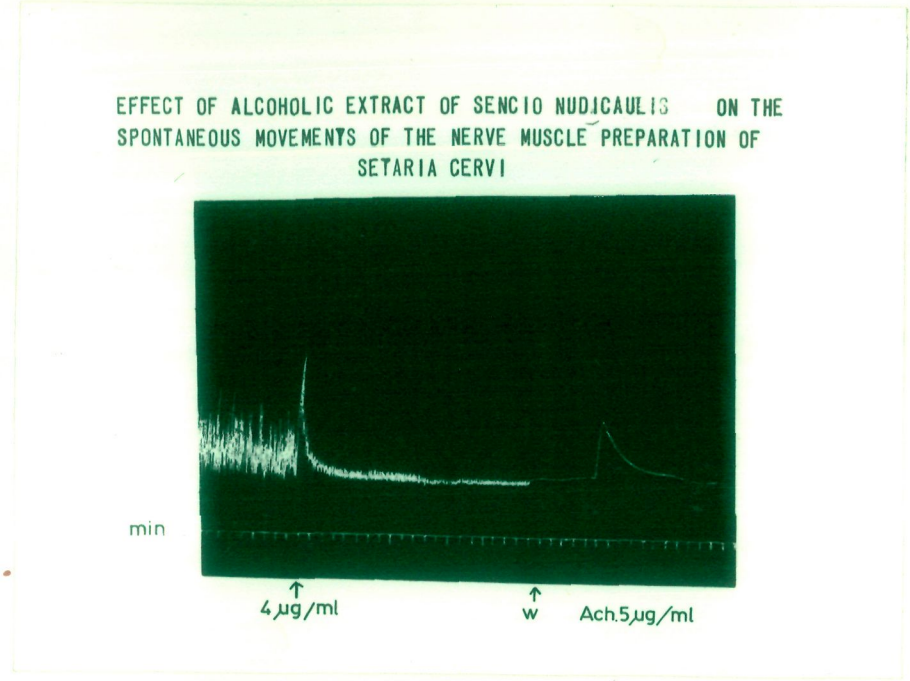


Fig. 8: Effect of alcoholic extract of the leaves of *S. nudicaulis* on the spontaneous movements of n.m. preparation of *S. Cervi.* A concentration of 4 µg/ml caused initial stimulation followed by irreversible paralysis. Repeated washings failed to restore the movement (w). Ach (5 µg/ml) produced its usual stimulant effect during the phase of paralysis.

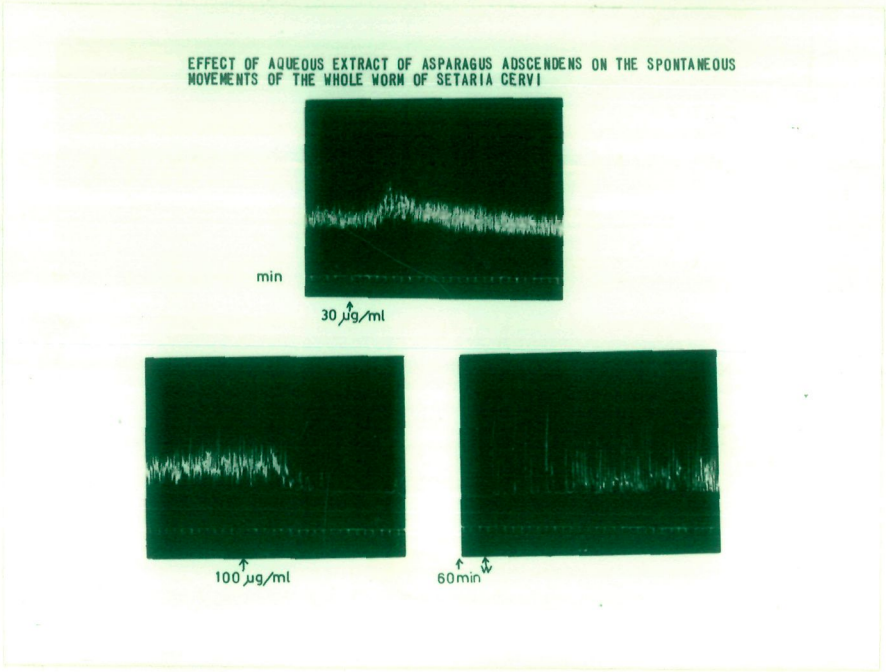


Fig. 10: Effect of aqueous extract of the roots of *A. adscendens* on the spontaneous movements of whole worm of *S. cervi.* A concentration of 30 µg/ml caused an immediate increase in amplitude, tone and rate of contractions.The effect on the tone was short-lived (upper panel). At a higher concentration of 100 µg/ml the stimulant effect of drug was characterized by short-lasting small increase in tone and amplitude followed by reversible paralysis (lower panel).

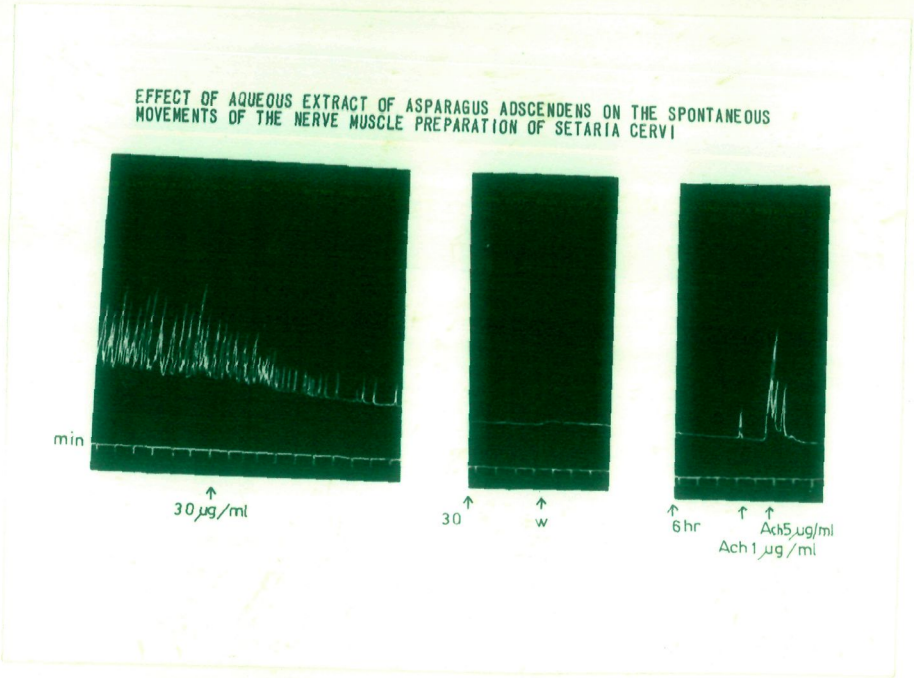


Fig. 11: Effect of aqueous extract of the roots of *A. adscendens* on the spontaneous movements of n.m. preparation of *S.cervi*. A concentration of 30 µg/ml caused decrease in amplitude, tone and rate of contractions followed by irreversible paralysis. The response to Ach was concentration related being more with a concentration of 5 µg/ml as compared to 1 µg/ml.

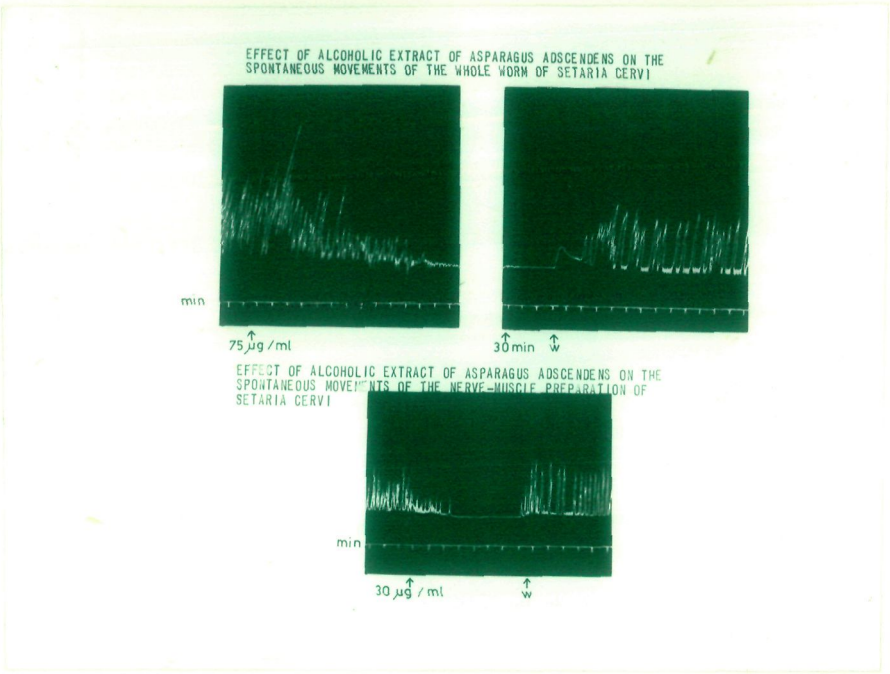


Fig. 12: Effect of alcoholic extract of the roots of *A. adscendens* on the spontaneous movements of whole worm (Upper panel) and n.m. preparation (lower panel) of *S. cervi*. A concentration of 75 µg/ml produced irreversible paralysis of whole worm while only 30 µg/ml was required to paralyse n.m. preparation.

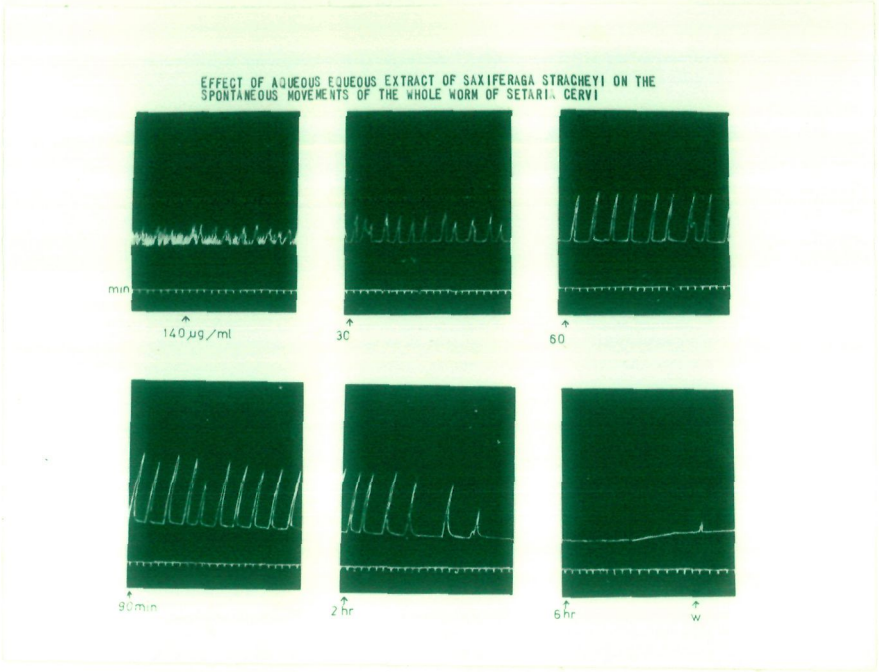


Fig. 14: Effect of aqueous extract of the roots of *S. stracheyi* on the spontaneous movements of whole worm of *S. cervi*. Bath applied concentration of 140 µg/ml caused initial stimulation characterized by increase in amplitude followed by irreversible paralysis. With the increase in amplitude the rate of contractions showed a corresponding decrease while the tone remained visibly unaffected.

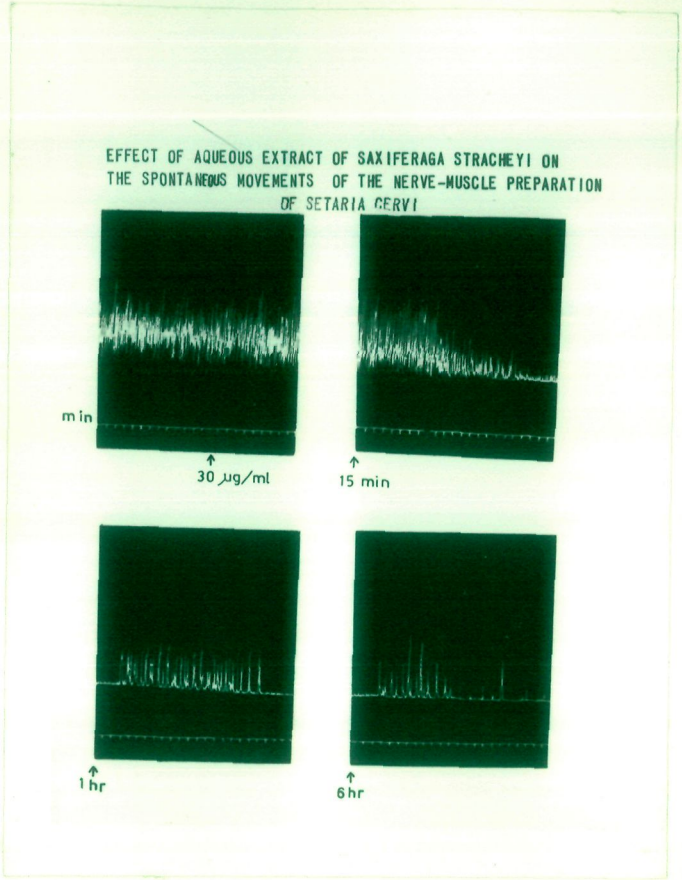


Fig. 15: Effect of the aqueous extract of the roots of *S. stracheyi* on the spontaneous movements of n.m. preparation of *S. cervi*. A concentration of 30 µg /ml caused an initial short-lasting stimulation followed by irreversible paralysis. After about 25 min the amplitude started decreasing and became lower than the pre drug level. During the whole process the tone and rate of contractions remained nearly unaffected.

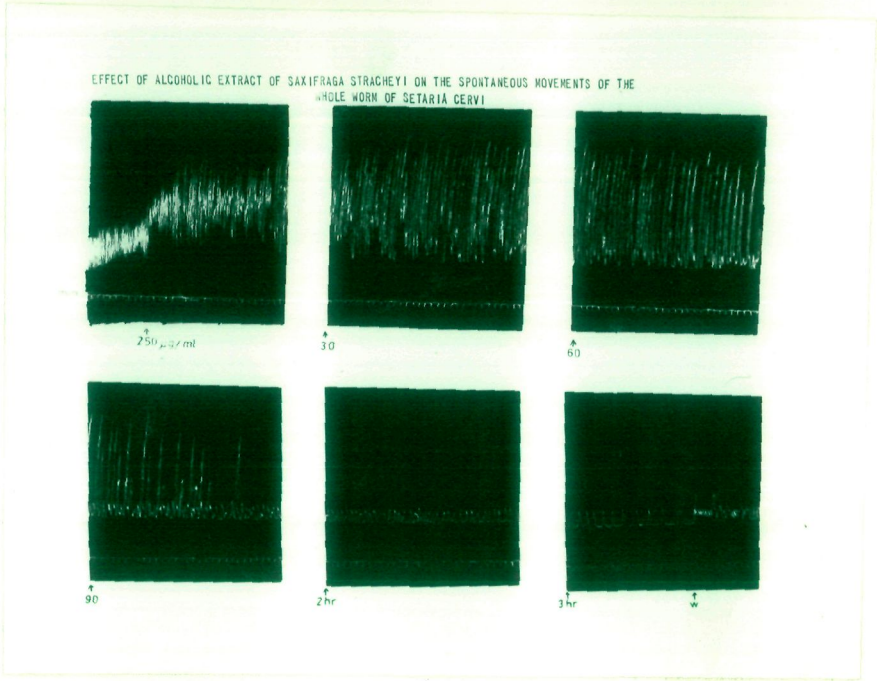


Fig. 16: Effect of alcoholic extract of the roots of *S. stracheyi* on the spontaneous movements of whole worm of *S. cervi*. Bath Applied concentration of 250 µg/ml caused stimulation characterized by increase in amplitude and tone of contractions followed by reversible paralysis. With the increase in amplitude the rate of contractions showed gradual decrease.

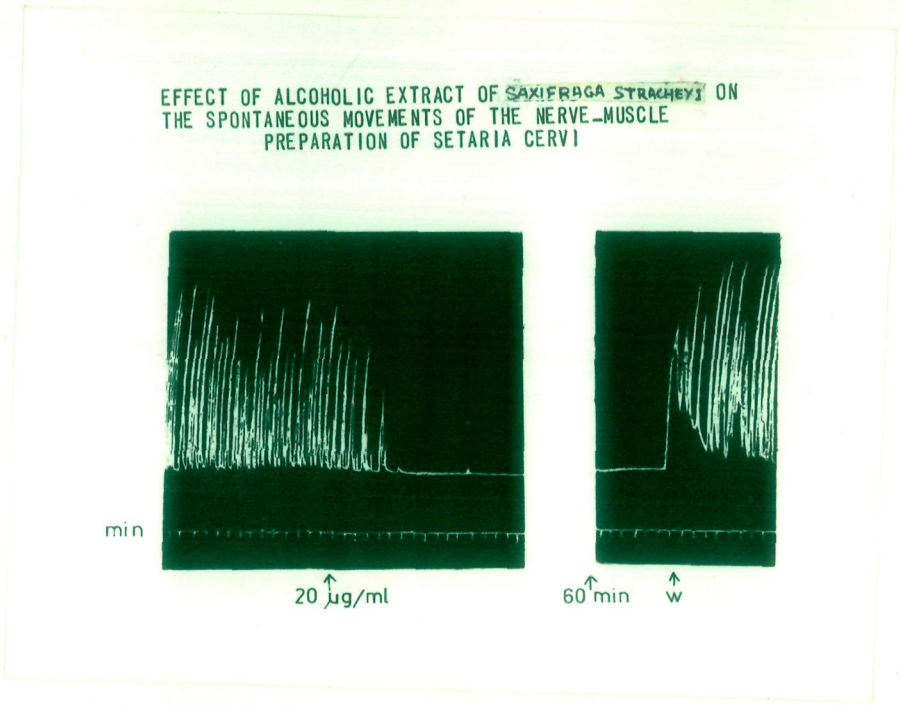


Fig. 17: Effect of alcoholic extract of the roots of *S. stracheyi* on the spontaneous movements of n.m. preparation of *S. cervi*. A concentration of 20 µg/ml caused reduction in rate and amplitude of contractions the tone remained visibly unaffected. The effect was reversible as repeated washing with bathing fluid restored the movements.

DISCUSSION

The observations indicate that recording of spontaneous movements of whole worm and n.m. preparaton of *S. cervi* is easy, convenient and reliable method to observe the effect of drugs on muscle tone, rate and force of contraction. Such study not only provides information regarding lethal or paralysing effect of drugs but also gives an insight into the mechanism of action. Besides macro and microfilaricidal activity resulting from direct lethal effect, the drug may cause inhibition of enzymes, blockade of nutrient uptakes, reversible and irreversible paralysis of the parasite.

I. *M. philippensis*

M. philippensis extracts are effective in lower concentration on n.m. preparation as compared to whole worm. The major difference between the two preparation is that the cuticle does not act as a barrier in the n.m. preparation. This indicates that cuticle can reduce the penetration of the extracts of *M. philippensis* into intact filarids. Substances with low lipid solubility find it difficult to penetrate nematode cuticle. This has been shown for *Ascaris* cuticle[53] and *D. vitae*[54]. Thus the aqueous extract could cause paralysis of n.m. preparation in five times less concentration than that required for whole worm while for the alcoholic extract the concentration required was eleven times less. The observation indicates that aqueous extract has more lipid soluble substances as compared to the alcoholic extract. Therefore, a drug with high lipid solubility will have a rapid onset and high filaricidal activity on intact preparation. The onset of action of both aqueous and alcoholic extracts was quick and characterized by stimulation of worm movements reflected mainly as increase in amplitude. This initial stimulant effect was followed by paralysis.

A number of anthelmintics interfere selectively with neuromuscular transmission of the nematode parasite. The bath applied Ach produces stimulation of spontaneous rhythmic movements of *Setaria*, which can be partially blocked by α-tubocusarine and not by atropine[55]. The Ach receptors of *Setaria* are similar in nature[56] with *Ascaris* Ach receptors[57,58]. Levamisole and pyrantel are potent agonists of *Acaris* and *Setaria* Ach receptors[59-61]. On *S. cervi in vitro* levamisole and tetramisole produce stimulation of the movements of whole worm and n.m.preparation characterized by increase in tone only in low concentrations while at higher concentrations the stimulatory phase though increased in intensity is reduced in duration and is followed by paralysis from which the worm does not recover[61].

On whole worm and n.m. preparation both aqueous and alcholoic extracts porduced increase in amplitude and frequency of spontaneous contractions which is followed by reversible paralysis. The response though has some similarity but is different.The stimulatory phase is more prolonged and does not occur in the form of spike. Further, the paralysis that follows is reversible as against irreversible caused by levamisole and pyrantel pamoate. Being agonist of Ach. at nematode receptors, levamisole will potentiate the action of the former. It is natural to assume that during paralysis induced by levamisole the response of Ach shall further enhance the paralysis and its effect as stimulant shall not be manifest, while on the other hand, the extracts of *M. philippensis*, as appears, acts by different mechanism and the response to bath applied Ach during the phase of paralysis manifest (Fig. 1).

Nematode exhibit rhythmic vigorous movements which help to maintain and locate themselves in the environment. These movements can be modified

by neurohumors and anthelmintic.While Ach has been identified as excitatory neurohumor, GABA and 5-HT paralyse the worm[62]. Studies have shown the presence of these substances in parasites. Anthelminitc like piperazine mimics the action of GABA and brings about hyperpolarization of *Ascaris* and *Setaria* muscle cells and that is associated with relaxation[59,62-64]. This hyperpolarization in *Ascaris* has been shown to be modified by increase in Cl^- conductance and not due to blockade of Ach receptors. DEC, a piperazine derivative on the other hand produces initial stimulation followed by reversible dose dependent paralysis which results from antagonism of voltage sensitive K^+ conductance[65]. The aqueous and alcoholic extracts of *M. philippensis* also produce increase in amplitude and frequency of spontaneous contractions of both whole worm and n.m. preparation of *S.cervi*. This is followed by reversible paraysis which is evident only at low concentration (upper panel Fig.2). Further response to Ach could be elicited in both whole worm and in n.m. preparation when they were paralysed by bath applied, aqueous as well alcoholic extracts. Such response was similar to that produced by DEC on the whole worm and n.m. preparation of *S. cervi* [66].

II *Sencio nudicaulis*

Aqueous and alcoholic extracts of *S. nudicaulis* produced relaxation of spontaneous movements of whole worm and n.m. preparation of *Setaria*. The concentration required to produce an equivalent effect in n.m. preparation was 3 and 20 times less for aqueous and alcoholic extracts respectively as compared with whole worm, indicating that aqueous extract is likely to penetrate the cuticular barrier with ease as compared to the alcoholic extract.

The presence of Ach, the stimulation of whole worm and n.m. preparation[55] and the presence of cholinesterase[56] has led to the suggestion that Ach is the excitatory transmitter at neuromuscular junction in *S. cervi*. Extensive studies with other nematode like *A. suum* has assigned Ach a role of excitatory neurotransmitter [67,68]. The action of Ach is dependent upon the availability of Ca^{++} ions. In absence of calcium in the bathing fluid the spontaneous motility of both whole worm and n.m. preparation decreases and ceases altogether with the passage of time. Calcium channel blockers like nifedipine also cause decrease in spontaneous motility leading to paralysis of the whole worm as well as n.m. preparation[69]. Aqueous and alcoholic extracts of *S. nudicaulis* cause concentration related decrease in spontaneous movements leading to irreversible paralysis. Repeated changes in bathing fluid failed to restore the movements. The preparation were viable at this stage too as indicated by their response to Ach produced an excitatory effect on both whole worm and n.m. preparation at a stage when there were irreversible paralysis.

Both nifedipine and extracts (aqueous and alcoholic) of *S. nudicaulis* cause irreversible paralysis of whole worm and n.m. preparation of *S. cervi*. The former blocks the stimulant effect of Ach while extracts of *S. nudicaulis* does not. The evidence suggest that the mechanism of action of the two is different. *S. nudicaulis* does not produce its effect either by blocking the ionic channels for calcium or by blocking Ach receptors.

S. cervi like *Ascaris* responds to GABA with hyperpolarization leading to relaxation and paralysis of whole worm and n.m. preparation. Anthelmintic piperazine[62] acts as a weak GABA agonist[65] and so also its derivative DEC used to treat brugian and bancroftian filariasis. The response of both extracts

on whole worm and n.m. preparation of Setaria was similar to DEC which was characterized by initial shortlived stimulation followed by irreversible paralysis[66].

The aqueous as well as the alcoholic extract of *S. nudicaulis* affected the survival of microfilariae adversely *in vitro*. The concentration required to kill 50 and 90% of microfilariae within a period of 6 h being much less than that required to modify the spontaneous motility of the whole worm or the n.m. preparation. The observations indicate that the two extracts of plant are both macro and microfilaricidal *in vitro*.This differentiate the action of *S. nudicaulis* extracts and DEC, since the latter causes irreversible paralysis of the whole worm and n.m. preparation but does not kill the microfilariae *in vitro*[70-73].

III. *Asparagus adscendens*

Musculature of nematodes is involved in spontaneous rhythmical motility, intestinal functions, reduction and structural support. Disruption of musculature activity of the parasite with result in cessation of activity essential for maintaining bodily functions. Anthelmintics used have activity directed towards causing flaccid or spastic paralysis similar to that observed in skeleted muscles or other smooth muscles, the myogenic activity is modulatal by neurotransmitters. In case of *S. cervi* Ach has been identified as excitatory while GABA and 5-HT have been attributed an inhibitory function[55]. The flaccid paralysis may result from either blockade of Ach receptors or agonistic action on 5-HT and GABA receptors. On the other hand spastic paralysis may be brought about by persistent depolarization due to accumulation of Ach at the myoneural function as has been shown for levamisole and phyrantel pamoate[61]. Further it has been shown that entry of Ca^{++} is essential for triggering

stimulation of movements. In the absence of available Ca^{++} or by blocking ionic channels for calcium, blocks the stimulant effect of neurotransmitters like Ach and drugs on *Setaria*[69].

On whole worm of *S. cervi* both alcoholic and aqueous extracts of *A. adscendens* produced stimulation either initially or in low doses and this was followed by paralysis. Since the effect was not manifest when n.m. complex was exposed directly to the drug, it may be assumed that the initial stimulant response was due to irritant effect of the drug. Such type of response has been with other substances as well, which cause irritation to the worm.

The outer cuticle prevents a permeability barrier to the drug. Thus the response to the drug is manifest at lower concentrations in n.m. preparation as compared to the whole worm. The permeability barrier varies widely with different drugs. For example, the barrier for Ach[56], GABA[55], DEC[66], piperazine[62], mebendazole[74] and levamisole[61] is 1/1000, 1/20, 1/100,1/10 and 1/5th respectively. On the other hand the barrier is absolute for 5-HT as modification of movements is not seen on whole worm at very high concentrations.

The cuticular permeability barrier for aqueous and alcoholic extracts of *A. adscendens* is small as the paralysant effect on n.m. preparation is manifest in nearly 1/3 rd concentration required to inhibit the movements of the whole worm. The effect of both extracts on whole worm and n.m. preparation was reversible as repeated flushing the bath restored the movements. The irreversible paralysant effect of aqueous extract on n.m.preparation indicates that the extract is different from alcoholic, and contains substances which has irreversible effect.

The effect of aqueous extract does not seem to be related to the action

of calcium channel blockers, as the stimulant effect of Ach could be elicited during paralysant phase. Should this action be like that of GABA or 5-HT, receptor binding studies may provide some clue.

On microfilariae *in vitro*, extracts of *A. adscendens* produced lethal effect at very low concentrations. Even the microfilariae which survived showed reduction in motility.

IV. *Saxifraga stracheyi*

It is interesting to note that the effect of alcoholic extract on both whole worm and n.m. preparation is reversible whereas aqueous extract produced irreversible paralysis of *Setaria* preparations. It is likely that either the ingredients extracted in water and ethanol are different resulting in differential activity of the two extracts or some else additional compound is extracted in aqueous phase which imparts irreversibility to the paralysant effect. Conclusive evidence can only be provided when different substances are isolated from the extracts and studies individually as well as in combination on the spontaneous motility of whole worm and n.m. preparation of *S. cervi*.

Whole worm movements are stimulated initially by both aqueous and alcoholic extracts whereas such effect on n.m. preparation is only observed with aqueous extract. On n.m. preparation alcoholic extract produces only paralysis without initial stimulation. Had the initial stimulant effect been only on the whole worm and not on n.m. preparation, suggestion could have been made that it could arise due to irritant effect on the cuticle as observed with the substances like DEC[66], mebendazole[74], 5-HT[55] and carbachol[55]. Another possibility could have been that the stimulant effect results from the action on cirum-oesophageal ganglia which is in place in the whole worm, cut off

alongwith anterior ½" in the n.m. preparation. This may be attributed to the effect of alcoholic extract but not for aqueous extract where initial stimulation is seen in n.m. preparaton as well, although the nature of stimulant response may be different (Fig.15).

Bath applied GABA and 5-HT produces hyperpolarization of both whole worm and n.m. preparation of *Setaria*, thus causing flaccid paralysis[62]. Electrophysiological studies with *A. suum* muscle have shown that piperazine acts as a GABA agonist of low potency on extra synaptic GABA receptors of the bag, mediating an increase in Cl^- conductance.Thus piperazine produces a dose - dependent and reversible increase in input conductance associated with a hyperpolarising potentials. DEC on the other hand does not react with GABA receptors but antagonised a voltage sensitive K^+ conductance[65]. DEC produces a similar response on *Setaria*, low doses cause stimulations characterized by increase in amplitude followed by paralysis[66]. The response of root extracts of *S. stracheyi* is similar to the effect of DEC on the whole worm and n.m. preparation of *Setaria*. The stimulant effect is more evident with aqueous extract on both whole worm and n.m. preparation. Should the effect of *S. stracheyi* root extracts be like DEC and the active ingredient producing the effect is different, it may provide another tool to combat filariasis or a chemical lead for synthesis of new derivatives which might prove to be potential antifilarial agents.

On the microfilariae of *S. cervi* extracts of *M. philippensis, S. nudicaulis, A. adscendens* and *S. stracheyi* reduced the survival time in a concentration related manner. If these concentration can be presented to the microfilariae *in vivo*, the extracts could be a useful tool for the tratment of filariasis. However, investigation on *in vivo* model are essential before making such a claim.

EXPERIMENTAL

I. Collection of *Setaria cervi*

S. cervi has been shown to bear close similarity to human filarial worms in its response to drugs[11,12,75]. The present study was designed to observe the effect of aqueous and alcoholic extracts of *Mallotus philippensis, Sencio nudicaulis, Saxifraga stracheyi* and *Asparagus* adscendens on the spontaneous movements of the whole worm and nerve - muscle (n.m.) preparation of *S. cervi*, a filarial worm of cattle [62, 55], and on the survival of microfilariae *in vitro*. Adult female *S. cervi* were collected from the pertioneal cavity of freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing modified Ringer's solution[76] (NaCl 9g, KCl 0.42g, $NaHCO_3$, 0.5g, $CaCl_2$ 0.24g, glucose 0.25g in 1l distilled water) at 37°C. The time period between the removal of the worms from the host to the laboratory was less than 5 h . In the laboratory, the worms were given repeated wash with the same solution to free them from any extraneous material.

II Whole worm preparation

Adult *S. cervi* were suspended in an isolated organ bath of 20ml capacity, in modified Ringer's solution at 37°C. Spontaneous movements of the worm were recorded on a slow moving drum[62]. Aeration was not required as it did not improve the motility of the worm. At least 15 min were allowed for a worm to settle down before adding an extract[55].

Body wall of S. cervi is formed by cuticula, subcuticula and musculature. When a drug is added to the bathing fluid, it should cross the cuticle and subcuticle to reach the underlying layer of muscular cells. It is likely that

musclar cells with nerves are sites of action of drugs affecting the n.m. complex, once they have crossed the cuticular and subcuticular barrier.

III Nerve - muscle preparation

A worm was placed in a petri dish containing modified Ringer's solution (37°C). Two dissecting needles were inserted into the worm at one end and the cuticle ws split longitudinally. The intestine and the uterus were severed at both ends and removed. The anterior 1 cm of the worm was removed to eliminate the influence of the nerve ring and cephalic ganglia. The remaining part was tied at either end and suspended in an isolated organ bath, containing modified Ringer's solution at 37°C. This preparation served to expose the n.m. complex directly to the action of the drugs and also could exhibit spontaneous rhythmical movements similar to those of the intact worm. After 15 min when the preparation was stabilized to elicit spontaneous movements, the drugs were added to the organ bath.

IV. Collection of microfilariae

The uterus of female *S. cervi* was cut at its junction with the vagina and just below the bifurcation, and removed from the worm. The uterus was teased with a needle in the solution and microfilariae were freed.The microfliariae were suspended in a human serum - Ringer mixture, the count was adjusted to 100ml, and 0.5 ml aliquots of microfilariae suspension were placed in sterilized screw cap bottles containing aqueous or alcoholic extract in equal serum: Ringer mixture (v/v). The extract was added in doubly increasing concentration of 5 ng/ml. The bottles were kept in an incubator at 37°C and examined under a microscope after 6 h, to count the living and dead microfilariae. The LC_{50} and LC_{90} was calculated from a concentration- death graph.

V. Extraction of plant material

The plant material was collected from foot hills of Himachal Pradesh and was identified by Dr. R.P. Singh (Scientist) Regional, Research Centre ((Ayurvedic), Mandi (H.P.).

The plants and part of plant used for extraction is indicated in table VI.

Table VI : Preliminary antifilarial screening of Indian medicinal plants on n.m. preparation of *S. cervi* at a concentration of 500 µg/ml.

S.No.	Plant	Plant Part	Activity of extracts	
			Aqueous	Alcoholic
1.	*Mallotus philippensis*	Leaves	+	+
2.	*Rhododendron arboreum*	Leaves	–	–
		Flowers	—	—
3.	*Sencio nudicaulis*	Leaves	+	+
4.	*Rumex hastatus*	Leaves	—	—
		Stem bark	—	—
5.	*Asparagus adscendens*	Roots	+	+
6.	*Ajuga parviflora*	Leaves	—	—
7.	*Murraya koenigii*	Leaves	—	—
8.	*Saxi fraga stracheyi*	Roots	+	+
9.	*Gerbera lanuginosa*	Roots	—	—

(+) active, (-) inactive.

In a typical procedure of extraction of plant material, 100 g of ecah plant was defatted with petroleum ether and extracted with 500 ml of ethanol twice. The combined ethanol extract was concentrated in vacuum and the residue was used for testing. The plant material was then extracted with 500ml of distilled water. The water extract was concentrated in vacuum and the residue was used for testing.

For preliminary screening of antifilarial activity with the residues obtained from ethanol and water extracts, weighed quantity of residue was dissolved in respective solvents (ethanol or water) and made to a concentration of 500 μg/ml. For fruther studies the solutions were diluted adding more solvents to get the solutions of a particular concentration.

REFERENCES

1. Wilson, T. *Trans. Roy. Soc. Trop. Med. Hyg.* ***1950***, 44, 49

2. Denham, D.A. and McGreevy, P.B. *Advances in Parasitology* ***1977***, 15, 243.

3. Rogers, R. and Denham, D.A. *J. Helminth.* ***1976***, 50, 21.

4. Williams, H.E. *Parasitology* ***1955***, 45, 56.

5. Scott, J.A.; Stembridge, V.A. and Sicley, N.M. *J. Parasitol.* ***1947***, 33, 138.

6. Hawking, F. *Experimental Chemotherapy* ***1963***,1.

7. Edeson, J.P.B. and Liang, A.B.G. *Ann.Trop. Med. Parasit.* ***1959***, 53, 394.

8. Mak, J.W.; Zaman, V. and Sivanandam, S. *Am. J. Trop. Med. Hyg.* ***1974***, 23, 369.

9. O'Holohan, D.R. and Zaman, V. *J. Trop. Med. Hyg.* ***1974***, 77, 113.

10. Nelson, G.S. *J. Helminth.* ***1962***, 34(3), 297.

11. Singhal, K.C.; Chandra, O.; Chawla, S.N. ; Gupta, K.P. and Saxena, P.N. *J. Pharm. Pharmacol.* ***1969***, 21,118.

12. Singhal, K.C.; Chandra, O. and Saxena, P.N. *Jap. J. Pharmacol.* ***1972***,22, 175.

13. Hawking, F. *J. Trop. Med.* ***1940***, 43, 204.

14. Hawking, F. and Swell, P. *Br. J. Pharmacol.* ***1943***, 3, 285.

15. Culbertson, J. and Rose, H. *J.Pharmacol. Exp. Therap.* ***1944***, 81, 181.

16. Friedheim, E.A.H. *Annales del la Societe Belge de Medicine Tropicals* ***1962***, 41, 367.

17. Townson, S. *Tropical Med. Parasitol.* ***1988***, 39, 475.

18. Peters, L.; Bueding, E.; Valk, A.D.; Higashi, A. and Welch, A.D. *J. Pharmacol. Expt. Therap.* ***1949***, 95, 212.

19. Hawking, F.; Ormerd, W.E.; Thurston, J.P. and Webber, W.A.F. *Br. J. Pharmacol.* ***1952***, 7, 494.

20. Hewitt, R.I.; White, E.; Wallace, W.S.; Stewart, H.W.; Kushner, S. and Subbarow, Y. *J. Lab. Clin. Med.* ***1947***, 32, 1304.

21. Hewitt, R.I.; Kushner, S.; Stewart, H.W.; White, E.; Wallace, W.C. and Subbarow, Y. *J. Lab. Clin. Med.* ***1947***, 23, 1314.

22. Campbell, W.C.; Fisher, M.H.; Stapley, E.O.; Albers, S.G. and Jacob, T.A. *Science* ***1983***, 221, 823.

23. Aziz, M.A.; Diallo, S.; Larviere, M.; Diop, I.M.; Porta, M. and Gaxotte, *P. Lancet* ***1982***, 1456.

24. Diallo, S.; Larviere, M. and Diop, I.M. *Bulletin de la Societo de Pathologic. Ex.: et: ac: Des: Fil.* ***1984***, 77(2), 196.

25. Taylor, H.R. and Greene, B.M. *Am. J. Trop. Med. Hyg.* ***1989***, 41(4), 460.

26. Greene, B.M.; Taylor, H.R. and Cupp, E.W. *The New England J. Med.* ***1985***, 313(3), 133.

27. Parveen, N.; Singhal, K.C.; Khan, N.U. and Singhal, P. *Indian J.Pharmacol.* ***1989***, 21, 16.

28. Wang, P.Y.; Chen, J.T. and Liu, L.H. *Chinese J. Parasitic Disease Control* ***1989***, 2(4), 264.

29. Parveen, N.; Khan, N.U. and Singhal, K.C. *Phytotherapy Research* ***1989***, 1, 1.

30. Srivastava, M.C.; Singh, S.W. and Tewari, J.P. *Indian J. Med. Res.* ***1967***, 55, 746.

31. Gupta, S.S.; Verma, P. and Hishikar, K. *Indian J. Physiol. Pharmacol.* ***1984***, 28, 63.

32. Nadkarni, A.K. *Indian Materia Medica*, Popular Book Depot, Bombay, Vol. 1, ***1954***; pp. 152, 761.

33. *The Wealth of India, Raw Material,* CSIR Publication, New Delhi, Vol. VI, **1962**; p. 229.

34. Chopra, R.N.; Nayar, S.L. and Chopra, I.C. *Glossary of Indian Medicinal Plants,* CSIR Publication, New Delhi, ***1956***; pp. 28, 160, 225.

35. Maheswari, J.K.; Singh, K.K. and Shah, S. *Bull. Med. Ethnobot. Res.* ***1980***, 1, 318.

36. Cromble, L.; Green, C.L. and Whiting, D.A.*J. Chem, Soc. (C)* ***1968***, 2625.

37. Ahluwalia, V.K.; Sharma, N.D.; Mittal, B. And Gupta, S.R. *Indian J. Chem.Sect. B.* ***1988***, 27B (3) 238.

38. Dean, F.M. *Naturally Occuring Oxygen Ring Compounds*, Butterworths Publication, London, ***1963***;p. 228.

39. Khorana, M.L. and Motiwala, D.K. *Indian J.Pharmacol.* ***1946***, 11, 37.

40. Widen, C.J. and Puri, H.S. *Planta Med.* ***1980***, 40 (3) , 284.

41. Merlin, L. *Corsi. Semin. Chim.* ***1968***, 11, 97.

42. Bandopadhyay, M.; Dhingra, V.K.; Mukerjee, S.K.; Pardeshi, N.P. and Seshadri, T.R. *Phytochemistry* ***1972***, 11 (4) , 1511

43. Aghoranurti, K.; Rajagopalan, T.R. and Seshadri, T.R. *Current Sci.* ***1964*** 33(3), 80.

44. Ahmed, M. *Pharmazie* ***1993***, 48 (1), 69.

45. Asolkar, L.V.; Kakkar, K.K. and Chakre, O.J. *Glossary of Indian Medicinal plants with Active Principles,* CSIR Publication, New Delhi, Part I, ***1992***; pp. 100, 122.

46. Sharma, S.C.; Chand, R. and Sati, O.P. *Phytochemistry.* ***1982***, 21(8), 2075.

47. Sharma, S.C.; Chand, R.; Bhatti, B.S. And Sati, O.P. *Planta Med.* ***1982***, 46 (1), 48.

48. Sharma, S.C. and Sharma, H.C. *Phytochemistry* ***1984***, 23 (3) , 645.

49. Tandon, M.; Shukla, Y.N. and Thakur, R.S., *Phytochemistry* ***1990***, 29 (9), 2957.

50. Sharma, S.C.; Chand, R. and Sati, O.P. *Pharmazie* ***1980***, 35(11), 711.

51. Hayashi, K.; Yamaki, T. and Inoue, T. *Acta Phytochim.* ***1949***, 15, 49.

52. Yamaki, T. and Inoue, T. *Misc. Repts. Res. Inst. Nat. Resources* ***1946***, 10, 13.

53. Fetterer, R.H. *Comp. Biochem. Physiol.* ***1986***, 84A, 461.

54. Christ, D.; Goebel, M. and Saz, H.J. *Br. J.Pharmacol.* ***1990***, 101 (4), 971.

55. Singhal, K.C.; Madan, B.R. and Saxena, P.N. *Indian J. Med.Res.* ***1977***, 66, 517.

56. Singhal, K.C.; Zehra, N.; Singhal, U. and Saxena, P.N. *Indian J.Physiol. Pharmacol.* ***1978***, 22, 71.

57. Baldwin, E. and Moyle, V. *Br. J. Pharmacol.* ***1949***, 4, 145.

58. Natoff, I.L. *Br. J. Pharmacol,* ***1969***, 37, 251.

59. Aubry, M.L.; Cowell, P. ; Davey, M.J. and Shevde, S. *Br.J. Pharmacol.* ***1970***, 38, 332.

60. Eyre, P.*J. Pharmac. Pharmacol.* ***1970*** , 22, 26.

61. Singhal, K.C. and Saxena, P.N. *Indian J. Pharmacol.* ***1978***, 10 (4) , 309.

62. Singhal, K.C.; Madan, B.R.; Saxena, P.N. and Johri, M.B.L. *Indian J.Pharmacol.* ***1975***, 7, 22.

63. Del Castillo, J.; DeMello, W.C. and Morales, T. *Arch. Int. Physiol. Biochem.* ***1963***, 71, 741.

64. Del Castillo, J.; DeMello, W.C. and Morales, T. *Experientia* ***1964***, 20, 141.

65. Martin, R.J. *Br. J.Pharmacol.* ***1982***, 77, 255.

66. Singhal, K.C.; Madan, B.R. and Saxena, P.N. *Indian J.Physiol. Pharmacol.* ***1978***, 22, 93.

67. DeBell, J.; Del Castillo, J. and Sanchez, V.*J.cell Comp. physiol.* ***1963***, 62, 159.

68. DeBell, J.T. *Q.Rev. Biol.* ***1965***, 40, 233.

69. Singhal, K.C.; Ganguly, B. and Awasthi, P.K. *Indian J. Physiol.Pharmacol.*

1995, 39, 117.

70. Oliver-Gonzales; Santiago-Stevenson, D.and Malonado, J.F. *J. Am. Med. Ass.* ***1949***, 139, 308.

71. Hawking , F.; Sewell, P. and Thurston, J.P. *Br. J.Pharmacol.* ***1950***, 5, 217.

72. McGregor, I.A.; Hawking, F. and Smith, D.A. *Br. Med. J.* ***1952***, 2, 908.

73. Taylor,A.E.R. *Trans. Roy. Soc.Trop.Med. Hyg.* ***1960***, 54, 450.

74. Singhal, K.C.; Madan, B.R. and Saxena, P.N. *Indian J. Pharmacol.* ***1978***, 10, 163.

75. Singhal, K.C.; Chandra, O. and Saxena, P.N. *Jap. J. Pharmacol.* **1972**, 22, 726.

76. Singhal, K.C. ; Saxena, P.N. and Johri, M.B.L. *Jap. J. Pharmacol.* **1973**, 23, 793.

LIST OF PUBLICATIONS

1. Two isomeric benzodipyranone derivatives from *Calophyllum inophyllum.*

   N.U.Khan, N. Parveen, M.P. Singh, Rajinder Singh, B. Achari, P.G.P. Dastidar, and P.K. Dutta

   Phytochemistry, **1996**, 42(4), 1188-83.

2. *In vitro* antifilarial activity of *Sencio nudicaulis* Buch. Ham. - Effect on *Setaria cervi* (Nematoda : Filarioidea).

   Rajinder Singh, N.U. Khan and K.C. Singhal

   Indian J. Physiol. Pharmacol. **1996**, 40(3), 231-36.

3. Potential antifilarial activity of the roots of *Asparagus adscendens* Roxb. against *Setaria cervi* (Nematoda : Filarioidea) *in vitro.*

   Rajinder Singh, N.U. Khan and K.C. Singhal

   Indian J. Exp.. Biol. **1996**, In Press.

4. Potential antifilarial activity of *Mallotus philippensis* (Lam.) : Studies on the whole worm, nerve-muscle preparation and microfilariae of *Setaria cervi* (Nematoda : Filarioidea).

   Rajinder Singh, N.U. Khan and K.C. Singhal

   Indian J. Physiol. Pharmacol. **1996**, In Press.

5. Exploration of antifilarial potential and possible mechanism of action of the root extracts of *Saxifraga stracheyi* on cattle filarial parasite *Setaria cervi.*

   Rajinder Singh, K.C. Singhal and N.U. Khan

   Phytotherapy Res. (Communicated)

PAPERS PRESENTED AT CONFERENCES/SYMPOSIA :

1. Constituents of *Calophyllum inophyllum.*

   N. Parveen, R. Singh and N.U. Khan

   Proc. 80th Ind. Sc. Cong. Part III **1993**, 71.

2. Search for HIV-inhibitory compounds : Structure and absolute configuration of a new benzodipyranone derivative and its isomer from the leaves of *C. inophyllum.*

   N.U. Khan, N. Parveen, M.P. Singh, R. Singh,B. Achari and P.K. Dutta

   Global Challenges in Drug Development **1994**, 27.

PAPERS UNDER PREPARATION :

1. The NMR studies of carbazoles from *Murraya koenigii.*

   R. Singh, N.U. Khan and M. Niwa, 1977

2. The constituents of *Gerbera lanuginosa.*

   R. Singh, N.U. Khan and M. Niwa, 1977